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The Journal of Allergy

VOL. 26 JULY, 1955 NO. 4

Original Articles

HISTAMINE: ITS PHARMACOLOGY AND ROLE IN ANAPHYLAXIS

CARL A. DRAGSTEDT, PH.D., M.D., CHICAGO, ILL.

THE pharmacology of histamine is an oft-told tale. There is not much that is new with which to intrigue you, and I can only hope that your repeated exposure to it has not rendered you hypersensitive.

As a prelude to my remarks, I would like to call attention to the fact that Dale's papers, which constitute so much of the foundation of our knowledge, have recently been assembled from the diverse journals in which they appeared and republished under the title of *Adventures in Physiology*.[1] I am sure that many of you have quoted other authors' quotations from Dale without consulting the original publications. These important papers are now readily accessible and are enlivened by some current commentaries that do much to revitalize them.

Histamine is a powerful and versatile agent, affecting a great many organs and tissues. Its major effects are those which are manifested upon the blood vessels, upon various organs and structures containing smooth muscle, and upon various glands.

In discussing its vascular action, it is probably best to describe its action upon capillaries as partially distinct from that upon other vessels. It is the most potent dilator of capillaries that is known. The dilatation is unique in that it is accompanied by a more or less parallel increase in capillary permeability which distinguishes it from some other dilator agents. The action appears to be directly upon the contractile mechanism and is independent of innervation as well as of the intermediary effect of any known humoral agent. The dilatation per se does not appear adequate to acount for the increased permeability, but the precise explanation of this is not clear. The increased capillary permeability is manifest in a variety of ways, by edema, by increased formation of lymph, by transudation of colloid dyes out of the blood stream, etc.

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The action of histamine upon the arterioles is more complex. For most species of animals, when histamine is perfused through isolated arteries or through the vascular bed of isolated organs, it appears to exercise a vasoconstrictor effect. This is what one would expect upon the basis of its known ability to contract smooth muscle. Nevertheless, in intact animals the action is more complicated. In the cat and dog, and in fact in most animals, it produces a fall in blood pressure which is associated with an increase in volume of most of the viscera except the kidney. There seems little doubt that there is some dilatation of the smaller arterioles. Dale has attempted to explain the discrepancy between the effects in the intact animal and in the isolated system as being due to the maintenance, under conditions of normal circulation, of a tone of the minute vessels, which histamine relaxes, whereas on somewhat larger arterial branches its action is one of pure vasoconstriction. A vasocontrictor effect with a rise in both pulmonary and systemic blood pressure is commonly observed in the rabbit, and Rocha e Silva[2] has reported that if one produces a shocklike condition by repeated intermittent occlusion of the pulmonary artery, histamine regularly produces a rise of pressure. I believe it is fair to say that we do not know all that we should know about the vascular action of histamine. The blood pressure is an end result which may represent the algebraic sum of a variety of effects that need separate analyses.

With few exceptions, histamine stimulates the smooth muscle structures of nearly all mammals. This is its cardinal and most fundamental effect. It, too, is not understood in precise terms. It appears to be an exaggeration of tonus which is not associated with very much increase in oxygen consumption by the contracting muscle. The sensitivity of the muscle varies markedly between various species, as well as between various organs in the same species. The smooth muscle structures of rats and mice are markedly resistant, while those of cats and guinea pigs are markedly susceptible. Those of dogs and rabbits seem to be intermediary. Within a given species the uterus and bronchi are highly susceptible; the intestinal, urinary bladder, and arterial muscles are less so; and the gall bladder and iris are still less so. These generalizations require some modification. The bronchi of the guinea pig and man are much more susceptible than those of the dog, cat, and rabbit, for example. These differences are associated with the differences in the lethal action of histamine. As is well known, the guinea pig usually dies from asphyxia consequent to severe bronchospasm, the dog from hypotension and circulatory failure, and the rabbit from right heart failure consequent to intense pulmonary arterial constriction.

Histamine stimulates all the gastrointestinal glands, but its action upon the gastric glands transcends that upon the salivary glands, pancreas, and intestinal mucosa to such an extent that the latter effects may almost be disregarded. Histamine provokes a large output of gastric juice which is more acid than that produced by a test meal. The mechanism of this action is again not known. It acts equally well after denervation of the gastric mucosa, and its action is inhibited only very slightly by atropine or by the antihistaminics. The gastric response is a very delicate one in that it can be provoked by a subcutaneous dose

of histamine which is so small as to produce no detectable change in the blood histamine. Of considerable interest is the fact that the gastric response to histamine is neither readily fatigable, nor does any substantial tolerance to it develop.

Histamine also provides an increased output of epinephrine from the adrenal medulla. I do not know whether it has yet been established whether there is any disparity between epinephrine and nor-epinephrine in this regard. This phenomenon has interest in connection with the reports that epinephrine acts to liberate histamine, and furnishes ammunition for those who believe that these antagonistic agents have a regulatory action on the circulation and tend to counterbalance each other to some extent.

There are other actions of histamine, such as its hyperglycemic effect, its ability to provoke as well as alleviate some headaches, etc., but these are all more or less subservient to the major effects described.

At the first meeting of the American Academy of Allergy in 1944 I presented a paper on the significance of histamine in anaphylaxis.[3] There is not a great deal that needs changing in discussing that subject today. I stated that the bill of indictment against histamine consisted of three chapters. The first chapter deals with the plausibility of its participation; the second, with those studies which have identified it in association with anaphylaxis; and the third consists of the confirmatory studies indicating that antihistaminic measures have antianaphylactic effects which are consistent with each other. These three chapters can be filled in briefly as follows:

The plausibility of the involvement of histamine in anaphylaxis consists of two categories of facts. The first of these is that histamine is present in substantial amounts in the organs and tissues which participate in the anaphylactic reaction. Suffice it to say here that conspicuous quantities are found in the dog's liver and in the guinea pig's lungs, which everyone agrees are entitled to be referred to as "shock organs" in these animals, and also in the rabbit's blood which I believe should be considered a shock organ in this animal. It is high in the skin of those animals, including man, in which skin reactions are common. Conversely, it is low to virtually absent in those tissues, such as the striated muscles, in which anaphylactic reactions are negligible. The second aspect of the plausibility of histamine's involvement lies in the fact that there is no known action of histamine that is inconsistent with any of the phenomena of anaphylaxis. It is true that there are a number of anaphylactic phenomena which cannot be accounted for by the known actions of histamine, but this is no inconsistency. It only means that we must look beyond histamine for the explanation of these. As is well known, a number of other agents serving to explain many of these have been detected and identified. Among these are heparin, bradykinin, the slow-reacting substance of Kellaway, and perhaps acetylcholine and others.

The second chapter of incriminating evidence regarding histamine consists of the numerous experiments in which histamine has actually been detected as being liberated during the anaphylactic reaction. The first of these experiments

was reported in 1932 and since then they have been repeatedly confirmed and extended. I believe there is no need to review them for this audience. There are few who would deny these well-established facts. There are some who view the liberation of histamine as the consequence of the anaphylactic reaction, having no etiological relationship to any of the events that transpire, and believe that we must look elsewhere for the responsible agent. This is just as illogical as it would be to assert that the incoagulability of the blood is unrelated to the heparin which is discharged. It has been shown that histamine is liberated in adequate amounts, that the time relationship of its liberation is precedent to the effects which it could provoke, and that by nullifying the effects of histamine one nullifies some components of the anaphylactic reaction.

The third chapter in the chain of studies which link histamine with anaphylaxis consists of those experiments purporting to challenge the histamine hypothesis by inducing anaphylactic reactions in animals which were supposedly protected against histamine. I say "supposedly" advisedly. You will recall that there were at least two procedures that supposedly would protect against histamine, which failed to protect animals against anaphylaxis and which also failed in the clinic. I refer to the use of histaminase (which as you will learn should be called *diamine oxidase*) and to the use of histamine-azoprotein. Now, there was never any substanial evidence that either of these, when administered in the manners then employed, were materially effective against histamine. Nevertheless, these failures were heralded as defeats for the histamine hypothesis, largely, I suppose, from some sort of uncritical conclusion that they ought to have worked. When the antihistamines were discovered, however, agents which had an unquestioned and easily demonstrated ability to protect against histamine, the situation was vastly different. These agents have marked ability to protect against or mitigate anaphylactic shock in practically all animals. Their use has served to bring out rather sharply those aspects of the anaphylactic reaction which are not accounted for by histamine, although this statement needs some qualification in that the antihistamines do not nullify all the actions of histamine, either qualitatively or quantitatively.

In summary, I would like to say that the thesis that histamine has a major role in the production of the anaphylactic phenomena in certain animals is firmly established. We cannot, as yet, delineate as sharply as we would like the precise boundaries between the histamine and the nonhistamine components of the reaction, but I have confidence that these gaps will be filled satisfactorily.

I am reluctant to conclude without asking you to reflect upon the meaning of this relationship of histamine to anaphylaxis. What useful end is being served, or what is nature's purpose, in having histamine discharged in this malevolent fashion? I confess to being something of a teleologist, with a belief that a highly complex reaction such as this must be the result of some evolutionary journey which has meaning. It is easy to say that it is idle to speculate. I do not hold with such a view. Speculation begets hypothesis, hypothesis begets experiment and study, and these beget knowledge and understanding. I challenge you to formulate some concept which may start such a chain reaction. I would then

urge you to have the temerity to present your ideas, for ideas beget ideas, and as much can often be learned in the process of proving them to be false as in proving them to be true.

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THE QUANTITATIVE DETERMINATION OF HISTAMINE IN ANIMAL TISSUES

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THIS article is intended mainly as a survey of the present status of chemical methods for the quantitative determination of histamine in animal tissues. Bioassay methods will be considered only briefly. Reference will be made primarily to the methods which now appear most useful, and, with apologies for many omissions of the earlier work, no attempt will be made to trace the chronological development of histamine assay methods in general.

Histamine assay, either chemical or biologic, usually requires that the histamine from an animal tissue first be purified to some extent. The histamine purification is omitted by some workers using bioassay and working with tissues quite rich in histamine. The most successful purification procedures for the chemical determination of histamine are briefly as follows: (1) By the method of Lowry, Graham, and their colleagues,[1] the tissue (or tissue extract) is treated with trichloroacetic acid which precipitates most of the proteins and large polypeptides. Histamine is adsorbed from the trichloroacetic acid solution on Decalso. This is accomplished most conveniently by allowing the solution to flow through a column of Decalso. The histamine is removed from the Decalso by a small amount of 40 per cent potassium bromide at 60 to 70° C. (2) The histamine purification procedure of McIntire and associates[2] consists of extracting the tissue with butanol, removing the histamine from the butanol by means of a cation exchange material, and finally eluting the histamine from the ion exchanger with dilute hydrochloric acid. When this method was developed there were no suitable cation exchangers available commercially; a very satisfactory cation exchanger, cotton acid succinate (which is just an insoluble polycarboxylic acid) was made specifically for the histamine purification procedure. Now that carboxy cation exchange resins are commercially available, some workers use them instead of cotton acid succinate.[9]

The butanol-cotton acid succinate purification was employed by Urbach and Giscafré[4] as a preliminary to paper chromatography for the qualitative identification of the histamine released from dog liver and from rabbit blood-formed elements during anaphylaxis.

Of these two histamine purification procedures, the Decalso method perhaps accomplishes a higher degree of purification, while the butanol-ion exchange method is more suitable if subsequent procedures such as paper chromatography are desired. Furthermore, samples purified by the butanol-ion exchange method may be assayed biologically, as well as chemically.

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There are now available two different types of chemical methods for histamine assay. Most of the older chemical assays for histamine have been based upon the coupling of histamine with a diazotized aromatic amine to produce a red-colored azo dye. The dye, after purification by simple extraction procedures, can be measured spectrophotometrically.[5-10] The position of coupling on the histamine molecule is not known; in fact, the colored product may be a mixture of histamine derivatives. A number of different aromatic amines have been used, but the most recent and apparently most successful methods of this type have employed p-nitroaniline as the amine to be diazotized.[8-10] Within the last few years, two new chemical methods for histamine assay, both employing 2, 4-dinitrofluorobenzene (DNFB) as the reagent, have been developed independently in our laboratory[3] and by Lowry and his co-workers.[1] The two methods are identical in principle, but different in manipulation. Histamine reacts with 2, 4-dinitrofluorobenzene under specified conditions to yield N^{α} -(2, 4-dinitrophenyl) histamine, a yellow-colored compound which absorbs light intensely, with an absorption maximum between 355 and 360 mμ.[3] This derivative is separated from excess reagent and other impurities by simple extraction steps, and the amount of derivative is then determined spectrophotometrically at 358 mμ. In the method of McIntire and associates[2, 3] the final volume of solution for spectrophotometric measurement is 1 ml., and 0.5 μg. of histamine can be determined satisfactorily. Graham and his associates carried out their final manipulations on a much smaller scale (0.05 ml.) and thus were able to measure as little as 0.01 μg. of histamine. The Lowry and Graham method may be operated more conveniently with larger samples when the amount of histamine is sufficient.[11]

When one considers their relative merits, the DNFB method appears to have the following important advantages over the azo dye method: DNFB yields a perfectly stable colored histamine derivative, a fact which is very important for the convenience of the method. The DNFB method can be interrupted for a period of hours or days at almost any point without jeopardizing the results. The azo dye method, on the other hand, yields an unstable colored derivative which at best deteriorates about 7 per cent in the first hour.[8] The stability of the derivative makes the DNFB method more convenient and more satisfactory, particularly where a large number of determinations are to be carried out simultaneously. The DNFB method also has an advantage in sensitivity; the published results indicate that, for comparable accuracy, the azo dye method requires at least five times the minimum amount of histamine that can be measured by the Lowry and Graham method. The DNFB method is also potentially the more specific of the two types of chemical methods. At present, both types appear adequately specific for the determination of histamine in the animal tissues investigated; however, the chemical nature and the stability of the DNFB derivative allow additional manipulations, which might be desirable for greater specificity in special cases, for instance, cases where the patient or the experimental material has had various drug treatments prior to the analysis for histamine. From what is known of

the chemistry of the two reactions, the DNFB method has a much smaller number of potentially interfering substances among compounds known to occur in the body and drugs which might be used.

Our knowledge of the chemistry of the DNFB method affords a prediction as to just what types of compounds could interfere, and both variations of this method are designed to eliminate all interfering substances known to occur in animal tissues. There are two types of compounds represented by many drugs which could cause trouble, and special manipulations might be necessary to eliminate their interference. They are: (1) tertiary amines where one R group absorbs light intensely in the 358 mμ region (for example, 8-hydroxyquinoline) and (2) compounds which have a tertiary amino group and also a primary or secondary amino group capable of reacting with DNFB (for example, 2-[2′-amino ethyl] pyridine or imidazoles other than histamine with a primary or secondary amino group on a side chain).

Let us now consider briefly the bioassay methods and the purification of histamine for bioassay. Numerous methods of purifying histamine have been used, most notable of which are ultrafiltration,[12] dialysis,[13] electrodialysis,[14] paper chromatography,[4, 13] the well-known method of Barsaum and Gaddum[15] which was modified by Code,[16] and the butanol extraction-cotton succinate procedure which was discussed earlier.[2] Of these, the butanol extraction-cotton succinate method accomplishes the greatest degree of purification, is the most convenient to use (particularly for large numbers of samples), and is least likely to allow undesirable changes in the extract during the process of purification. The Barsoum-Gaddum and Code procedures, by far the most widely used of all, were designed more to destroy interfering substances than to accomplish an actual purification of histamine. Recent reports indicate that the Barsoum-Gaddum procedure, with or without the Code modification, may result in high histamine results because of the actual formation of histamine by the decarboxylation of histidine.[17, 18] Depending upon the relative amounts of histamine and histidine present, this source of error might be serious.

The biologic assays for histamine are based upon the extent to which histamine will (a) cause a strip of isolated guinea pig ileum to contract, or (b) cause a drop in the blood pressure of the cat.

A piece of guinea pig ileum is suspended in Tyrode's solution in a small glass chamber which is mounted in a constant temperature bath. The piece of ileum is anchored firmly to the glass chamber on the lower end and is suspended at the upper end by a thread which is tied to one end of a lever arm. Contractions of the ileum strip are recorded on the kymograph. Theoretically, the amplitude of contraction is proportional to the concentration of histamine in the solution in which the ileum is suspended. There are a number of designs of the apparatus, some with provisions for automatic filling and emptying of the chamber in which the piece of ileum is suspended.[19, 20]

For assay by the cat blood pressure method, the cat is anesthetized and set up for recording of the carotid arterial pressure.[21] The histamine solution

usually is injected into the femoral vein. The drop in blood pressure, recorded on the kymograph, should be proportional to the amount of histamine injected—within certain limits.

When a very small (for example, 2 ml. volume) bath is used for the guinea pig ileum, it is about twenty times as sensitive as the cat assay and may be preferred where such sensitivity is necessary. In our laboratories, the cat assay has been found to be the more reliable and more easily controlled, and it is preferred over the guinea pig ileum when there is sufficient histamine in the samples to be assayed.

In choosing between chemical and bioassay methods, in the past the guinea pig ileum method has been preferred because of its sensitivity. This is no longer a valid reason, because the DNFB method of Lowry and Graham now offers essentially the same sensitivity with greater precision. There are, on the other hand, a number of good reasons for preferring the chemical methods:

1. Chemical methods are more reliable, more controllable and are always ready to go. Often one has difficulty in finding a cat that will be suitable. Sometimes one spends considerable time getting the guinea pig ileum to "settle down." Once one has the bioassay set up, there is no assurance as to how long it will remain under control. The bioassays are very capricious; frequent restandardization of the cat or the muscle strip is always necessary.

2. After one has "tooled up," the chemical methods require less work than the bioassays and are much more suitable for handling large numbers of samples.

3. The DNFB chemical method offers one the possibility of almost unequivocal qualitative identification as well as quantitative determination on the same sample, if such is desired.

4. In the recent work with synthetic histamine releasers, the chemical method will tell the difference between histamine and the releaser; the bioassay method may not.

In spite of the obvious advantages of the chemical methods, many prominent workers in histamine resarch have been reluctant to give up the guinea pig ileum assay.

To one who is planning a project which will require histamine assays, the DNFB chemical method should be recommended as first choice. Whether the version of Lowry and Graham, or the method from our laboratories should be used would depend upon the circumstances. If less than 0.5 μg. of histamine is to be measured, Lowry's method should be employed. All materials used in the Lowry and Graham method are commercially available, and therefore this method might be preferred for assaying a moderate number of samples. Although the method from our laboratories requires some special materials

which must be made "at home," the actual routine operations are in some ways easier and, for large numbers of assays over a period of time, the extra "tooling-up" may be worth while.

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A METEOROLOGIC APPROACH TO THE HAY FEVER PROBLEM

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I. THE SIGNIFICANCE OF THE HAY FEVER PROBLEM

THE seriousness of the disease called "hay fever" is readily demonstrated by an estimation of its magnitude: hay fever and asthma stood fourth in prevalence in a 1935-36 survey list of chronic diseases.[1] About 2 to 4 per cent of the entire population of the United States suffers from hay fever,[2] and the prevalence has increased steadily each year.[3] Furthermore, approximately 40 per cent of those who suffer from hay fever eventually develop the more serious malady of asthma, according to early surveys.[4, 5] Entirely aside from the probability of extended or permanent disability, the economic importance of a few days' to several weeks' annual discomfort and partial disability due, for example, to ragweed pollen sensitivity in three million people is considerable.

II. HISTORY OF THE PROBLEM

The literature on this subject dates back to Blackley[6] and to the complaints of Daniel Webster, quoted by Barker,[7] but the complexity of the problem has not been sufficiently appreciated until quite recently. Scheppegrell,[8] who first introduced a physical approach to pollen research, has been attacked by a number of writers[9-11] for the mistakes he made in applying Stokes's law, but his critics failed to achieve a real solution because they made important mistakes of their own. The most usual error is oversimplification. The general assumption that the fall rates of pollen grains in still air are directly representative of their behavior in moving air is an example. What is not clear to the nonmeteorologist is that the air near the ground is often very turbulent during summer days, and that this turbulence frequently is more important than gravity in determining the motions of small particles.

Wodehouse[12] has expressed the view that the study of hay fever is the province of "the clinical and the botanical" specialists. His viewpoint appears to be exceptional, but probably none of the biologic group expresses the need for meteorologic participation as appropriately as Stakman[13] does. The meteorologists, on the other hand, have tended to find other interests more pressing, but not without exception. Bergeron,[14] in commenting upon a misinterpretation of meteorologic possibilities in the transport of *Aloina* spores, presents an analysis of the problem of transport of particles in the atmosphere. Hewson[15] has included a concise review of the pollinosis problem

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in his discussion of atmospheric pollution, and Dingle[16] has presented suggestions for the more adequate understanding of pollen counts in relation to simultaneous symptoms.

III. THE ROLE OF THE WEATHER

Almost invariably, the writers in this field have sought to relate the observed phenomena to the weather. That there exist some relationships is obvious; what the relationships are is more obscure. Scheppegrell[8] observed, "It is well known that hay-fever patients improve during a rain of several days' duration, and that their symptoms become aggravated during the prevalence of high winds. . . . It has been frequently reported that hay-fever is aggravated by hot weather, but the records for the past year indicate that high temperatures have no special effect. On the contrary, the records . . . show that most of the severe stages of hay-fever developed . . . when the temperature was unusually low for the season. . . . The lower temperature, however, was due to the high wind which blew from the northeast. . . ." He then incorporated maximum and mean wind speeds, wind direction, mean temperature, and amount of rainfall with his pollenometric counts. Durham[17] was also frustrated by the complex interactions of weather phenomena, pollen development and release, and clinical symptoms. Correlations between air temperatures and pollen counts were, for his data, sometimes strikingly high and at other times very disappointing in support of the conclusion of Scheppegrell. His discussion of antecedent weather influences upon the development of the pollen source plants and of rain, wind, and humidity upon the release and transport of the pollen grains gives a good picture of the complexity of the total problem. In a later paper,[11] he has reported on the effect of extremes of humidity upon pollen densities. Hawes and his associates[18] devised a volumetric counting device and attempted to measure diurnal changes of volumetric pollen counts in Los Angeles, but they found no consistent diurnal pattern. More recently, Smith and Rooks[19] found that, on the average, airborne ragweed pollen grains were about ten times as plentiful during the day (6 A.M. to 6 P.M.) as during the night at Iowa City in the ragweed season, Aug. 7 to Sept. 20, 1952. These writers, together with many who have preceded them, have overlooked the fact that their averages characterize in a very limited way only the period and place that they studied. The important feature of the weather that seems uniformly to be omitted from consideration is its extreme variability. That is to say, for example, never before was there a season exactly like the 1952 season at Iowa City, nor will there ever be again. The point that requires emphasis is that relationships of the pollen counts to specific weather events and atmospheric conditions are required. These events and conditions are approximately repeated and, by prediction and interpretation of their relationships to atmospheric pollen incidence, effective guidance for the allergist and his hay fever patients is possible. To choose an extreme example, it is entirely possible that a season characterized by frequent nocturnal cold front passages and cool, calm days at Iowa City

could seriously alter, if not completely reverse, the figures presented by Smith and Rooks. It is true that the atmosphere tends, on the average, to be more unstable (that is to say, more prone to convection and turbulence) during the day than during the night, but frontal systems, circulation patterns, and the larger-scale atmospheric disturbances progress on a time scale independent of the period of the earth's rotation. Since these phenomena are especially effective in lifting the pollen grains into the air in great numbers and in carrying them to great heights and extreme distances, one should not expect pollen counts or symptoms to be completely controlled by diurnal influences.

The Production of Ragweed Pollen.—Long before the hay fever season begins, the weather exerts its impact upon the plants. Durham[17] states that ". . . rainfall is by far the most important" factor influencing the annual pollen crop, and that "Its greatest effect is preseasonal." He reports some preliminary estimates: "Statistics seem to indicate that the amount of rainfall in July has more influence on the pollen crop than the rainfall of any other similar period. . . ." Brown[20] writes that the amount of rainfall in June, July, and August determines the size of the ragweed crop, just as the quantity of rainfall influences food crops. He also gives the rule: "the larger the plants the more pollen they produce." Wilmer and Cobe[21] emphasize the effects that the drought of 1930 produced in delaying the onset of the pollen season and the date of maximum atmospheric pollen counts. Durham[22] points out that this delay was quite general over the eastern part of the United States. The literature contains a number of such references, indicating a general agreement that the cumulative effects of antecedent weather must affect the pollen crop, but no quantitative criteria or estimates are to be found. Such criteria have been developed for purposes of predicting yields of a few farm crops,[23-25] suggesting by the analogy Brown[20] used that a quantitative solution for ragweed pollen predictions is also possible.

The Quality of the Pollen Crop.—In addition to the quantity of pollen released during the hay fever season, the factor of quality or chemical composition of the pollen is considered by many to be important in determining the severity of symptoms experienced. Wilmer and Cobe[21] found strong indication that the antigenicity of pollen varied from place to place and from season to season, and they suggested that the soil, the weather, and microbiologic influences needed to be considered in this regard. Specifically, the pollen season of 1930 ". . . produced a different type of pollen which exhibited more potency as a stimulant . . ." than that of earlier seasons. Cobe,[26] in response to Durham's[22] question, cited the following hypothetic case, based on experience: ". . . pollen collected . . . in a field . . . within the first two or three days of pollination . . . made up into extract may give us a three-plus skin test, and two or three days afterward there will be a severe rainfall and something has changed the pollen collected from the same . . . area, whose nitrogen content has not been altered very much. It may give only a one-plus or plus-minus reaction. Apparently something has happened to the potency of the pollen in that area . . ." which exhibits sufficient acuity of observation to be

significant. Furthermore, this observation is reasonably consistent with the analogous observations on crops. Salmon[27] writes, of cereal grains, that ". . . A marked deficiency of moisture during the latter part of the growth period . . . leads to higher protein content of grain . . . because of reduced photosynthesis and a lower yield." Here, then, is a plausible process whereby the protein content of pollen grains also may be modified. Hopkins[24] suggests another, again an observation on the response of wheat: ". . . temperature conditions during the period of formation and ripening of the wheat grain may affect . . . protein content. . . . This is presumably the result of an actual reduction in carbohydrates due to accelerated respiration . . . the rate of respiration (in wheat) is approximately doubled by each 10° C. rise in temperature." Peirce[28] reports a large variation, dependent upon weather conditions, of the vitamin content of forage crops. Kramer and his co-workers[29] have prepared a monograph on the influence of meteorologic factors on the yield and quality of barley. All these findings are significant, by analogy, as indicators of the probable role that weather plays in determining the severity or moderation of any particular hay fever pollen situation.

IV. POLLEN PREDICTIONS

That weather factors influence pollen productivity and antigenicity and that hay fever pollen in general depends upon the atmosphere for its transport and dispersion imply that an adequate evaluation of the influences of weather should make pollen predictions possible. Two distinct types of prediction are to be anticipated: a long-term, preseason prediction and a short-term, day-to-day prediction of relative pollen counts during the hay fever season. As is the case with all weather predictions, those for hay fever pollen must be made for specific areas or regions, that is to say, they cannot be generalized to cover the entire United States, or even one-half the nation, and still retain any real meaning. In a measure, this may be true, even of a single city, as regards day-to-day forecasts, particularly in the first few days of the pollen season.

Seasonal Forecasts.—Apparently the best cues with regard to long-term prediction of the hay fever season are to be found in the work which has been done on various agricultural crops. The solution of the pollen prediction problem should follow in similar lines. Dates for the most advantageous harvesting of canning peas have been determined for some years on the basis of the governing weather factors. Curry[30] presents data for staggering harvests of peas along the Atlantic Seaboard. Thornthwaite and Mather[31] present a comprehensive discussion of the research in progress at the Johns Hopkins University Laboratory of Climatology. Based upon seedbed and early growth conditions in May, and using climatic data and/or long-range forecasts for June and July conditions, the date of beginning of the ragweed season and the antigenicity and amount of pollen to be expected may be "foreshadowed."[7] As the season evolves, phenologic observations on the plants and cumulative weather effects may be applied to correct the initial estimates and thereby to

adjust and improve the prediction. This method should provide a high accuracy of prediction at least two weeks before pollen release begins. Such forecasts as these might apply to fairly extensive regions, such as New England, etc. Direct benefits to hay fever victims would evolve, but one immediate gain would be the use of such information for planning a pollen season vacation away from areas of anticipated high pollen counts in relatively mild seasons and entirely out of the ragweed areas for the bad seasons.

Day-to-Day Forecasts During the Season.—When the anthers have developed to the bursting point, the need for more specific information on the degree of pollen pollution and the pollen antigenicity becomes apparent. Furthermore, the general seasonal type forecast becomes nearly useless except as a rather flexible guide. At this point, the release of the pollen grains and their transport become a complex of specific phenomena in response to specific weather events.

It is well known that vigorous cold fronts, squall lines, and thunderstorms are capable of carrying large amounts of pollen to great heights and over long distances and that these events usually produce extremely high local pollen concentrations for a limited time. Such an occurrence cannot be averaged over a day or any other period of time, nor does the patient average the high pollen count out in his symptoms. The storm and the pollen storm are incidents, and should be thought of, predicted, and perhaps treated for as incidents.

More subtle than these storms is the weather development characteristic of the slow-moving, strong polar anticyclones that frequently traverse the northern half of the hay fever region. The initial event is the passage of a cold front with its associated squall and thunderstorm activity and extremely high pollen counts. Following this, the wind gradually dies and the fresh polar air, as yet quite free of pollen grains, rests coolly and quietly on the land, providing relief for the hay fever patient. A gradual cumulative warming of the air follows, usually over a period of two or three days, and as it does so, the ragweed unrelentingly produces its pollens. Toward the end of this period, the slightest disturbance of a ragweed plant will produce a visible shower of pollen. Undoubtedly, the pollen crop accumulates both in ripe anthers and in loose deposits on leaves and on the dry ground surface. Then the breeze, most often from the south or southwest, begins, slightly gusty at first and increasingly so as time passes. The pollen grains are borne aloft in ever greater numbers and to increased heights as the breeze develops, and the patient experiences progressively increasing discomfort. Continued intensification of the southerly wind, gustiness, and warming are precursors of a fresh cold front passage which frequently, but not always, will begin an approximate repetition of the sequence.

The continuous development of weather sequences such as that just cited is the key to their day-to-day predictability. It is not sufficient, as Scheppegrell,[8] Durham,[17] and many others have found, to take one weather element,

such as temperature, as the determining factor. The atmospheric concentration of pollen depends upon the whole complex of atmospheric conditions and events which we call "weather," and it can be consistently predicted within reasonable limits only by virtue of the continuity and predictability of the whole weather picture.

Thus, day-to-day prediction of atmospheric pollen concentrations is a specialized problem of forecasting, but one well within the grasp of the meteorologist whose understanding of the pollen problem is adequate and who has sufficient botanical guidance as regards the state and distribution of the source plants. Advantages of predicted pollen counts over the standard gravity slide counts would be at least threefold:

1. They would be available thirty-six to forty-eight hours before the gravity slides could be counted. This would make possible the anticipation of the degree of exposure which a patient should expect to encounter on the day following the forecast. Such information should be of greater value clinically than the day-old pollen counts available under the present system.

2. They usually would be more representative of the atmosphere overlying a whole city, for example, than the pollen count at any particular place would be. That is to say that, because of the ever-present local and micro-influences upon spot observations, which can and do fluctuate widely, the prediction based upon the anticipated state of the atmosphere and of the pollen sources would be a better estimate of the general level of pollen concentration in the air than any spot observation is likely to be.

3. They would include estimates of the time variation of pollen concentrations throughout the forecast period. Anticipation of reasonably specific periods of minimum and maximum pollen concentrations, with estimates of relative pollen counts, based upon predicted weather events and conditions, would provide for planning of the hay fever patient's activities during the forecast period.

V. SUMMARY

Meteorology is of primary importance in the problem of hay fever, as the atmosphere carries the allergens to the hay fever patients. This transportation function is accomplished in different ways, depending upon the states of the atmosphere and of the source plants: (a) certain weather *events* are known to produce very high pollen counts for short periods of time, and (b) certain weather *sequences* tend to produce an extended cumulative effect. Generally speaking, such processes as these are not properly evaluated by the use of time or space averages, or by the assumption of uniformity in time and/or space. The meteorologist with suitable botanical guidance is thus

placed in a position from which he can render substantial aid to the hay fever clinician. Potential contributions of the meteorologist to the problem include the following:

1. He can help to procure meaningful measurements of atmospheric pollen concentrations and to interpret them.

2. He can help to anticipate the degree of severity of the developing hay fever season by means of long-term predictions, progressively modified.

3. During the season, he can help to chart the day-to-day course of activity and treatment for the patient by means of specialized daily predictions based upon phenologic and meteorologic information.

The problem is of sufficient importance and complexity to justify a coordinated attack by meteorologic, botanical, clinical, and immunochemical specialists.

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TREATMENT OF POLLEN HAY FEVER AND ASTHMA WITH AEROSOLS OF POLLEN EXTRACTS

Introductory Observations

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ASTHMA has been produced in man and/or guinea pigs with nebulized allergens by Schiller and Lowell[1-5] and Herxheimer.[6-12] Herxheimer reported desensitization by aerosols of allergens in guinea pigs and man. Specific local desensitization of passively sensitized guinea pigs by aerosols of antigens and haptens has been studied in this laboratory.[13, 14] An attempt to treat pollen hay fever and asthma with aerosols of pollen extracts seemed to be a logical sequel to this experimental background.

This report is an analysis of the observations made on twenty-one adult patients treated with aerosols of pollen extracts. Ten were treated during the late summer of 1953. Three of these and eleven others were treated during the 1954 fall pollen season.

Although obviously introductory, the data presented are sufficiently clear-cut to leave little doubt that some cases of pollen hay fever and asthma can be treated effectively by aerosols of pollen extracts.

Apparatus and Materials.—A DeVilbiss No. 40, or other, nebulizer with a special attachment for nasal inhalation by hay fever patients, plus an adjustable flow of compressed air of about 5 liters per minute, is all that is needed. An electric room air compressor is satisfactory.

A pollen extract mixture of seven parts ragweed and one part each of goldenrod, cocklebur, and corn was used arbitrarily. This is used in injection treatment at this clinic. Stock 1:33 weight by volume extracts, made by extracting 3 grams of dry pollen with 100 ml. of buffered glycerosaline, were diluted with normal saline to provide the weaker concentrations noted in the case reports.

Precautions.—Syringes with needles attached, 1:1,000 aqueous epinephrine, ampules of aminophylline and antihistamines for intravenous administration, and a spare nebulizer preloaded with 1:200 Isuprel were at hand. A physician was in attendance at all times. The patients were required to remain for at least thirty minutes after each treatment. Antihistamines, aminophylline suppositories, and sympathomimetics to control delayed reactions were provided.

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Treatment Details.—Deviations from the following details, as shown in the tables, were dictated by the progress of the individual cases and by the absence of prior experience.

The scratch, intracutaneous, and conjunctival reactions were titrated with the pollen mixture to provide some indication of the degree of the patient's sensitivity. The concentration of the initial inhalation was selected arbitrarily for each patient, 1:400,000 or weaker. Case 6 (1953) and Case 10 (1954) were exceptions. In general, treatment was started with a weaker concentration than that which gave a faintly positive intracutaneous reaction; 1.0 ml. was finally found to be the most convenient volume to use for each treatment.

The transoral route of inhalation was used to treat asthma and the nasal route was used for hay fever. In the transoral route, the lips were closed around the nebulizer tip to insure inhalation of the full 1.0 ml. of extract. For the nasal route, both nares were plugged with the tips of the nasal attachment which comes with the DeVilbiss No. 40 nebulizer. Three to five inhalations were given initially, and the patient was observed for fifteen minutes. The remainder was then inhaled without letup unless hay fever or cough occurred. One to three treatments were given daily. Arbitrary increments in concentration were given at each treatment. If symptoms occurred, the dose was reduced.

The nose should be inspected for patency before nasal treatments are given. When obstructed, the nares should be opened by bland decongestant sprays.

In the 1953 season, six patients received all treatments in the laboratory. Four other patients were allowed to take inhalations from a DeVilbiss No. 40 hand-pumped nebulizer at home, after fairly strong extracts were well tolerated in the laboratory. Compressing the bulb thirty times was shown to deliver about 0.1 ml. The daily dose at home was usually thirty to forty inhalations three to five times daily, or approximately 0.3 to 0.5 ml.

In 1954, all treatments were started in the laboratory. When improvement was noted, all fourteen patients were allowed to continue inhalations at home.

The results presented are based on periodic interviews and on postseason questionnaires. It is an old and familiar story that this method of interpreting results is fallible. For purposes of this report, it is unimportant whether an individual case or symptom was relieved 65 per cent, 75 per cent, or 85 per cent. It is important and accurate to state that all those who reported 65 per cent or more relief were highly pleased with the results.

Selection of Cases.—There were only three requirements: (1) hay fever and/or asthma limited to, or markedly accentuated during, the ragweed season; (2) positive skin and/or conjunctival reactions to the fall pollen extracts; (3) availability to the laboratory several times daily.

The ten 1953 cases are presented in some detail. The essential data on the 1954 cases are summarized in a table, plus some relevant comments.

1953 CASE REPORTS

Case 1 (Coseasonal Aerosol Alone).—The patient was a 39-year-old nurse with typical perennial hay fever accentuated markedly during the fall pollen season for twenty-three years. She was greatly relieved by injections of fall pollen plus other inhalant extracts from June, 1946, until she discontinued them early in the summer of 1952. However, she had little difficulty during the 1952 ragweed season. She was next seen on Sept. 1, 1953. Her hay fever had been troublesome for one week. There were strong skin and conjunctival reactions to the fall pollen mixture. She was treated by the aerosol schedule shown in Table I.

TABLE I. COSEASONAL INTRANASAL INHALATIONS

DATE	AMOUNT (ML.)	CONCENTRATION OF FALL POLLEN MIXTURE*	REACTION	COMMENTS
9/1/53 A.M.	0.5	1:400,000	None	
P.M.	0.5	1:40,000	None	
9/2/53 A.M.	1.0	1:40,000	None	
P.M.	1.0	1:20,000	None	
9/3/53 A.M.	1.0	1:8,800	None	90% Improved
P.M.	1.0	1:4,000	None	
9/4/53 A.M.	1.0	1:2,000	None	90% Improved
P.M.	1.0	1:1,100	None	
9/5/53 A.M.	1.0	1:800	None	90% Improved
P.M.	1.0	1:400	None	
9/6/53-9/9/53 Once daily	1.0	1:400	None	90% Improved
9/29/53-10/2/53 Once daily	1.0	1:400	None	90% Improved

*This is the ragweed concentration. The other pollen extracts in the mixture are weaker (see text).

Comment: The only therapy was aerosol desensitization. Relief was striking by the third day. There were no complications of the therapy. Antihistamines were not necessary. She estimated 90 per cent relief. Multiple perennial inhalant problems did not prevent excellent relief from aerosol therapy. The patient was treated successfully by aerosol again in 1954 (Case 3, Table VIII).

Case 2 (Coseasonal Aerosol Alone).—The patient was a 29-year-old physician with typical ragweed hay fever for nine years, but no asthma. When first seen on Sept. 2, 1953, symptoms were unusually severe and were becoming refractory to the antihistamines which had been effective previously. There was some obstruction from a deviated nasal septum. His skin and conjunctivae reacted strongly to the fall pollen mixture. Aerosol treatment was given as shown in the protocol (Table II).

Comment: Some relief was noted within forty-eight hours. Treatment was discontinued on Sept. 18, 1953, because he had no symptoms. An acute upper respiratory infection on Sept. 8, 1953, subsided soon after Gantrisin

was started. He wheezed a little after one aerosol treatment at the peak of the infection. The aerosol was omitted for three days. Symptomatic remedies were not needed after aerosol therapy was stopped. The deviated nasal septum did not prevent the good response to therapy. He estimated 100 per cent relief of rhinorrhea, pruritus, and nasal stuffiness and 75 to 80 per cent relief from sneezing. Relief seemed clearly due to aerosol therapy.

TABLE II. CASE 2. COSEASONAL INTRANASAL INHALATIONS

DATE		AMOUNT	CONCENTRATION OF FALL POLLEN MIXTURE*	REACTION	COMMENTS
					Symptoms ± 10 days
9/2/53		1.0 ml.	1:800,000	None	
9/3/53	A.M.	1.0 ml.	1:80,000	None	
	P.M.	1.0 ml.	1:40,000	None	
9/4/53		1.0 ml.	1:20,000	None	Definite relief
9/5/53-9/7/53			None		
9/8/53		1.0 ml.	1:20,000	None	A cold
9/9/53			None		
9/10/53	A.M.	1.0 c.c.	1:20,000	None	
	P.M.	1.0 c.c.	1:8,000		Mild wheeze in 6 to 8 hours
9/11/53		1.0 c.c.	1:8,000		Tight chest; pus in nose; started on sulfa
9/12/53-9/14/53		None			
9/15/53		1.0 ml.	1:8,000	None	Cold O.K.
9/16/53			None		
9/17/53		1.0 ml.	1:4,000	None	
9/18/53		1.0 ml.	1:2,000	None	No symptoms; no antihistamines needed

*See footnote, Table I.

Case 3 (Pre- and Coseasonal Aerosol Alone).—A 22-year-old woman had had severe hay fever and asthma in August and September and mild perennial hay fever from house dust for nearly twenty years. Benadryl and epinephrine, her only prior treatment, had been only partly effective. Preseasonal physical findings included a pale, boggy nasal mucosa, enlargement of the left inferior turbinate, and a few symptomless expiratory wheezes during forced expiration. There were positive scratch reactions to the fall pollens and plantain, plus many positive intracutaneous reactions to routine foods and inhalants. The fall pollen conjunctival tests were strongly positive in high dilution. Her aerosol treatment schedule is shown in Table III.

Comment: Aerosol treatment was begun about fifteen days before the onset of the ragweed season. It was continued coseasonally.

It was difficult to regulate the patient's aerosol dosage. There were some cough and purulent sputum for a few days at the end of August. She was given a supply of extract to use in the hand nebulizer one week before the pollen season. Two weeks after the pollen season began, she reported annoying asthma on two occasions only. In previous years, she had been uncomfortably asthmatic almost constantly at this stage of the season. Frequent stuffy nose but little sneezing were noted. The aerosol hand nebulizer treatments were continued. An occasional transient mild wheeze was the only sign of asthma during the last three weeks of the season.

Her postseason evaluation of aerosol therapy was 90 per cent relief or rhinnorrhea, nasal pruritus, and lacrimation. She estimated 80 per cent relief of the nasal stuffiness, sneezing, and sluggishness, and about 80 per cent relief of the asthma. She took antihistamine pills three or four times daily at first, but discontinued them about the middle of September.

Several reactions to the preseasonal treatments were severe enough to require one or two doses of Adrenalin. It is interesting that the early reactions

TABLE III. CASE 3. PRE- AND COSEASONAL TRANSORAL PLUS INTRANASAL INHALATIONS

DATE	AMOUNT	CONCENTRATION OF FALL POLLEN MIXTURE*	REACTION	COMMENTS
8/ 4/53 A.M.	0.25 ml.	1:40,000,000	None	
P.M.	0.25 ml.	1:4,000,000	None	
8/ 5/53 A.M.	0.25 ml.	1:400,000	None	
P.M.	0.25 ml.	1:40,000	Delayed wheeze	
8/ 6/53 A.M.	0.4 ml.	1:40,000	None	
P.M.	3.0 ml.	1:400,000	Delayed wheeze	
8/ 7/53	3.0 ml.	1:200,000	Delayed wheeze	
8/ 8/53	3.0 ml.	1:100,000	Delayed wheeze	
8/10/53 A.M.	3.0 ml.	1:50,000	None	
P.M.	3.0 ml.	1:25,000	Mild immediate wheeze	
8/11/53 A.M.	3.0 ml.	1:20,000	Mild immediate wheeze	
P.M.	3.0 ml.	1:8,000	Immediate wheeze and cough	
8/12/53	1.0 ml.	1:8,000	Immediate wheeze and cough	
8/13/53 A.M.	1.5 ml.	1:8,000	Immediate wheeze and cough	
P.M.	3.0 ml.	1:50,000	Immediate wheeze and cough	
8/14/53 A.M.	3.0 ml.	1:25,000	Immediate wheeze and cough	
P.M.	2.0 ml.	1:25,000	Immediate wheeze and cough	
8/15/53	3.0 ml.	1:30,000	None	Mild cough
8/16/53-8/28/53	20 inhalations 4 or 5 times daily	1:2,000	None	Hand nebulizer at home. No wheezing, mild cough with purulent sputum, hay fever improved 50%
	(August 21-22—Season started)			
8/29/53-8/30/53	30 inhalations 3 times daily	1:2,000	None	Occasional mild asthma
9/ 1/53-9/ 5/53	30 inhalations 3 times daily	1:2,000	None	Annoying asthma twice
9/ 5/53-9/30/53	30 inhalations 3 times daily	1:2,000	None	Mild daily wheeze

*See footnote, Table I.

to the weaker concentrations of pollen extract were delayed. When the concentration was increased, the reactions were prompt. Some of the reactions to pollen aerosol may have been intensified by the low-grade bronchitis a few days before the season began. The delayed and prompt reactions, in contrast to Herxheimer's[8-10] cases, did not necessitate interruption of aerosol treatment. Instead, the concentration of the inhaled extract was reduced markedly

after the second episode of delayed wheezing. The increments thereafter consisted of doubled doses instead of the greater increases given in other cases in which there were no reactions.

Her improvement seemed clearly the result of aerosol therapy, since no other treatment was used except antihistamines, which had been only partially effective before. Her polyvalent perennial allergy did not prevent good results from pollen aerosol treatment.

She was treated successfully by aerosol again in 1954 (Case 2, Table VIII).

Case 4 (Coseasonal Aerosol Plus Injections).—The patient was a 38-year-old white woman with typical severe hay fever starting Aug. 12, 1953, for the first time. She had been given daily injections of penicillin for one week and large doses of a variety of antihistamines, elsewhere, without benefit. There

TABLE IV. CASE 4. COSEASONAL INTRANASAL INHALATIONS PLUS INJECTIONS

DATE	AMOUNT	CONCENTRATION OF FALL POLLEN MIXTURE*	REACTION	COMMENTS
8/26/53-9/2/53	Coseasonal pollen injections given here			Onset of symptoms, August 12
8/26/53	0.25 ml.	1:400,000	None	Preliminary dose
		Aerosol program		
9/ 3/53 10 A.M.	0.5 ml.	1:400,000	None	
2 P.M.	0.5 ml.	1:40,000	None	
4 P.M.	0.5 ml.	1:20,000	None	
9/ 4/53 10 A.M.	1.0 ml.	1:20,000	None	
12 M.	1.0 ml.	1:8,000	None	
3 P.M.	1.0 ml.	1:4,000	None	
9/ 5/53-9/ 9/53	30 inhalations 3 times daily	1:1,000	None	Improved Hand nebulizer at home
9/10/53-9/17/53	30 inhalations 3 times daily	1:500	None	75% improved
9/17/53-10/ 2/53	40 inhalations 3 times daily	1:500	None	More improvement

*See footnote, Table I.

were purulent flecks in both nares, plus partial obstruction by a deviated nasal septum. Intracutaneous reactions were positive to ragweed and several other inhalants. Conjunctival tests with goldenrod and cocklebur pollen were strongly positive and precipitated acute hay fever within five minutes. Daily coseasonal injections of fall pollen extracts, started Aug. 26, 1953, plus symptomatic therapy, provided little relief in one week. Because of this, treatment with fall pollen aerosol was started on Sept. 3, 1953. She had been given a preliminary aerosol dose on August 26. The schedule of aerosol treatments is shown in Table IV.

Comment: Her postseason evaluation of aerosol therapy was 100 per cent relief from stuffy nose and lacrimation, 90 per cent relief of rhinorrhea, nasal

pruritus, and sluggishness, and 60 per cent reduction of sneezing. She continued to receive an injection of weak fall pollen extract once a week during the season.

The severe hay fever was not benefited by antibiotics (naturally), nor by antihistamines plus daily coseasonal injections of fall pollen extract. Improvement was noted on the fourth day of aerosol therapy and was estimated at 75 per cent relief within one week. Symptomatic remedies, which had been used until September 17, were not needed during the last two weeks of the season. There were no disagreeable effects from the aerosol.

Aerosol desensitization seemed to be the chief factor in her improvement, since seven days of coseasonal injections of pollen extract failed to provide relief. Coseasonal injections are usually effective within the first five days, if at all.[15] The possibility of improvement from the coseasonal injections cannot be dismissed, however.

Case 5 (Pre- and Coseasonal Aerosol Plus Injections).—The patient was a 26-year-old man with perennial hay fever and asthma, accentuated in grass and ragweed seasons. Complications were marked deviation of nasal septum, chronic sinusitis and bronchitis, emphysema, postpolio kyphoscoliosis, and funnel breast. Antihistamines and injections of grass pollen extract, elsewhere, had provided little relief. There were strongly positive skin reactions to the fall pollens plus numerous other inhalants. Conjunctival reactions were positive to the fall pollen mixture, grass, and plantain.

He was free of symptoms when preseasonal aerosol therapy was begun on July 29, by the schedule shown in Table V.

Comment: Reactions to aerosol inhalations were so troublesome the first two trials that they were discontinued both times. Five injections of fall pollen extract were given between August 7 and 21. No more injections were given.

Aerosol treatment was resumed, for the third trial, on August 13 with weaker extracts for two days. On August 16 the patient was given some extract to inhale daily from a hand nebulizer at home. On August 28 another treatment was given in the laboratory. The hand nebulizer was used from August 28 to September 4. By that time he had become essentially asymptomatic. On October 20 he reported only two mild attacks of asthma during September. One of these was precipitated by a typical respiratory infection. The pollen aerosol accentuated his asthma during the respiratory infection.

It seems fortunate that aerosol treatment was begun three weeks preseasonally. The difficulty in adjusting the dose was nearly solved by the beginning of the season. The possibility of Herxheimer's[8-10] hypersensitive state induced by reactions to aerosols must be considered here, but the reduced tolerance for antigen aerosols in the presence of bronchitis seems a more likely explanation for the reactions early in the course of aerosol therapy.

It is impossible to assess the role of aerosol in his improvement, estimated at more than 50 per cent, because he received some preseasonal injections of pollen extract.

TABLE V. CASE 5. PRE- AND COSEASONAL TRANSORAL INHALATIONS PLUS INJECTIONS

DATE	AMOUNT	CONCENTRATIONS OF MIXTURES		REACTION	COMMENT
		FALL POLLEN	GRASS AND PLANTAIN		
7/29/53 A.M.	1.0 ml.	1:8,000,000	1:2,000,000	None	
A.M.	1.0 ml.	1:800,000	1:200,000	None	
A.M.	1.0 ml.	1:80,000	1:20,000	Asthma in 5 hours	
7/30/53 A.M.	1.0 ml.	1:8,000	1:2,000	Immediate asthma	
P.M.	1.0 ml.	1:16,000	1:4,000	Asthma in 1 hour	
8/ 5/53	3.0 ml.	1:16,000	1:4,000	Immediate tight chest	
8/ 6/53	3.0 ml.	1:20,000	1:6,000	Immediate and delayed asthma	
8/ 7/53	Started fall pollen injections				
8/13/53	3.0 ml.	1:200,000	1:60,000	None	
8/14/53	3.0 ml.	1:50,000	1:15,000	Delayed wheeze	
8/16/53-8/30/53	30 inhalations 3 times a day	1:4,000	1:1,000	Hay fever and asthma, 2 nights	Hand nebulizer at home
8/21/53	Pollen injections discontinued				Season began
8/28/53	3.0 c.c.	1:20,000	1:6,000	Delayed wheeze	
9/ 1/53-9/30/53	30 inhalations 3 times a day	1:2,000	1:600		Occasional transient wheeze, 2 mild attacks
10/ 1/53-10/20/53	30 inhalations 3 times a day	1:2,000	1:600		O.K.

This case is presented in detail to illustrate the problems of adjustment of dosage, rather than as an example of striking improvement from aerosol therapy. It also shows that, with care, aerosol therapy is not contraindicated in complicated cases.

Case 6 (Coseasonal Aerosol Plus Injections).—The patient was a 44-year-old man with fall hay fever for twenty-two years and fall asthma for twelve years. He got satisfactory relief from coseasonal pollen injections in 1942 and 1943. Thereafter, his symptoms were controlled satisfactorily by symptomatic remedies until 1952, when hay fever and asthma became troublesome again.

Mild hay fever began on Aug. 17, 1953. There were positive intracutaneous and conjunctival reactions to the fall pollen mixture. Coseasonal fall pollen extract injections were started then. On Aug. 27, 1953, he complained of local reactions and abdominal pain after each injection. There were no reactions to five subsequent smaller daily doses. Hay fever had not

been relieved by coseasonal injections and antihistamines, but was worse when aerosol treatment was started on Aug. 27 by the schedule shown in Table VI.

Comment: Note that he was given a fairly strong vial of extract to take at home from a hand nebulizer. When he returned on Sept. 10, 1953, he was essentially symptom-free. Definite improvement was noted by September 1, three days after (1) starting aerosol, (2) reducing the coseasonal injection doses, and (3) changing antihistamines.

Through an error, a vial of strong fall pollen extract was given to him on September 10. A few inhalations of this caused immediate severe hay fever and moderately severe asthma. He recovered promptly. On September 13, 1:2,000 fall pollen mixture was started by aerosol at home. He had no more trouble during that season.

TABLE VI. CASE 6. INTRANASAL INHALATIONS OF COSEASONAL AEROSOL PLUS INJECTIONS

DATE	AMOUNT	CONCENTRATION OF FALL POLLEN MIXTURE	REACTIONS	COMMENTS
8/18/53- 8/27/53	(Coseasonal pollen injections daily)		Local and gastrointestinal	Onset of hay fever, August 17
		Aerosol started		
8/27/53	0.25 ml.	1:8,000	None	
8/28/53- 9/ 3/53	40 inhalations 3 times a day	1:4,000	None	Hand nebulizer
	(Smaller coseasonal pollen injections daily)		None	Better by September 1
9/ 3/53- 9/10/53	40 inhalations 3 times a day	1:4,000	None	90% relief; no antihistamines
9/11/53	"Few inhalations"	1:40	Immediate hay fever and asthma	
9/13/53-10/ 2/53	40 inhalations 3 times a day	1:200	None	No symptoms
10/ 2/53-10/10/53	40 inhalations 3 times a day	1:200	Aggravated the cough of acute respiratory infection	

The original plan was to have him continue the aerosol perennially. However, he developed an acute respiratory infection in October. Aerosol aggravated the cough markedly, so it was stopped.

He estimated 90 per cent relief of nasal stuffiness, pruritus, and sneezing. He had no asthma except after the inhalation of the concentrated fall pollen mixture.

This case suggests that aerosol may be an effective supplement to coseasonal injections of pollen extract. The patient had troublesome reactions and no relief after nine coseasonal injections of relatively weak extract plus antihistamines. There are the same problems of accurate appraisal of aerosol as in Case 4.

The chief reasons for including this case are: (1) the troublesome reaction to an inadvertent overdose of aerosol, (2) failure to develop the "hypersensitive state" of Herxheimer, and (3) marked aggravation of the cough of bronchitis after the season by the aerosol.

Routine perennial injections of fall pollen extracts were given until the 1954 season started, when he promptly developed severe hay fever. Aerosol therapy relieved him again in 1954 (Case 1, Table VIII).

Case 7.—The patient had polyvalent perennial hay fever and frequent respiratory infections plus fall pollen hay fever. Coseasonal treatment with injections of fall pollen extract plus dust had not helped. Supplementary aerosol treatment produced an estimated 25 per cent relief. The following winter was her best one for years.

Case 8.—Treatment of uncomplicated fall pollen hay fever by injections and aerosol immediately before and during the season provided 90+ per cent relief. There were no undesirable reactions to the injections or to the aerosol. Evaluation of aerosol versus injections as factors in the marked relief is impossible.

Case 9.—A 20-year-old woman with perennial and ragweed hay fever developed severe, acute bronchitis during the aerosol treatments. She developed asthma on the fifth day of treatment and refused further aerosol. This is a clear-cut example of failure and is another illustration of the aggravation of bronchitis by aerosol therapy.

Case 10.—A 51-year-old woman with perennial, grass, and fall hay fever and asthma had been well controlled for about ten years by perennial allergen injections. Early in the 1953 season, she developed severe hay fever in spite of continued injections of fall pollen extract, an air-filtered room, and large doses of antihistamines. Aerosols of fall pollen extracts seemed to provide marked relief within a week. Injection and aerosol treatments were continued. Later in the season, however, asthma developed for the first time in ten years. This coincided with a period of acute, severe, emotional stress. Emotional episodes had previously precipitated asthma. Fall pollen extract aerosol apparently relieved the hay fever but did not prevent what seemed to be psychogenic asthma.

COMMENTS ON THE FOURTEEN 1954 CASES

The 1954 cases were treated by a more uniform schedule than were the 1953 cases. The pollen mixture, precautions, technique of administration, and criteria for selection of cases were the same as in 1953.

The treatments were given by the technicians by the routine outlined in Table VII. The essential clinical data on the fourteen 1954 cases are summarized in Table VIII and in the following comments:

All the patients had hay fever. Five had asthma, but it was quite mild in four patients. All but two (Cases 3 and 11) were treated only co-seasonally with the aerosol. Those two patients had pre- and coseasonal aerosol therapy.

TABLE VII. 1954 ROUTINE FOR POLLEN AEROSOL TREATMENT

1. Sequence of aerosol strengths
   1—1:4,000,000 fall pollen (= ragweed concentration)
   2—1:400,000 fall pollen
   3—1:40,000 fall pollen
   4—1:24,000 fall pollen
   5—1:12,000 fall pollen
   6—1:4,000 fall pollen
   7—1:2,400 fall pollen
   8—1:1,200 fall pollen
   9—1:400 fall pollen
2. A treatment = 5 whiffs, 15 minutes' observation, then inhale rest of 1.0 ml.
3. Two or 3 treatments daily at ± three-hour intervals until relieved. Use above dose sequence.
4. Stop the inhalation at the first suggestion of tight chest, cough, wheeze, or hay fever.
5. Maintain relief by hand nebulizer.

Eleven patients obtained striking relief, estimated at 65 per cent or better. All the symptoms were relieved to about the same degree. One patient (Case 14) developed an obstinate virus infection of the upper and lower respiratory tracts about one week after starting the aerosol. It was impossible to evaluate the result of aerosol therapy.

Relief was usually apparent by the fourth day and maximal by the tenth day.

The initial concentrations of aerosols were weaker than those given in 1953, in general. 1:4,000,000 was the usual arbitrary starting dose. Patient 10 (who received striking relief) was the exception.

The final concentration varied. In each case it was approximately that concentration which was reached about the time that definite improvement was noted. This was the usual strength used at home.

All the 1954 patients completed treatment at home with a hand nebulizer. A few supplementary office treatments were given to three, in whom the hand nebulizer seemed not to maintain relief. In two of these, the over-all result was excellent.

There were no severe or prolonged reactions. Six patients had transient intensification of nasal symptoms either from the first few doses or, later, when stronger extracts were used. Four had mild bronchial reactions in the form of cough or wheezing.

All the patients had used antihistamines, with only fair relief, prior to treatment. During the last three weeks of the season, only six required symptomatic treatment. Of these six, one or two antihistamine doses per day provided almost complete relief to four patients.

Only one patient (Case 1) had had perennial or preseasonal injections of pollen extracts. He got no relief from them. Patient 7 was given some co-seasonal injections to attempt to increase the degree of relief from aerosol. No additional improvement was obtained from these injections.

Eight patients had had injections of pollen extracts in previous years. Seven felt that the results of aerosol therapy were better. One thought that the results were the same.

TABLE VIII. ESSENTIAL DATA ON AEROSOL TREATMENT, 1954 FALL POLLEN SEASON

CASE NUMBER	1*	2†	3‡	4	5	6	7§	8	9	10	11	12	13	14
Diagnosis: pollen asthma (all had hay fever also)	0	+	0	0	0	0	0	+	0	+	+	0	0	+
Date aerosol started	9/2	8/21	8/5	9/13	9/1	9/2	8/31	9/15	9/8	9/8	8/11	9/7	9/9	8/31
Relief started (number of days)	4	2	Pre-seasonal	1	2	1	3	3	1	3	18	3	3	2
Relief maximal (number of days)	10	5		5	5	20	10	8	2	6	30	7	7	3
Aero. route: per oral plus nasal (all used nasal route also)	0	+	0	0	0	0	0	+	0	0	0	+	0	+
Aerosol dilutions														
Initial = 1:(In millions)	4.0	4.0	0.4	4.0	4.0	4.0	1.2	4.0	4.0	0.04	4.0	4.0	4.0	4.0
Final = 1:(In thousands)	4.0	40.0	0.4	0.4	2.4	4.0	2.4	40.0	1.2	4.0	0.4	0.8	4.0	1.2
Hand nebulizer effective (all used hand nebulizer)	+	+	±	+	+	+	+	±	+	+	+	+	+	0
More laboratory aerosol needed	0	0	+	0	0	0	+	+	0	0	0	0	0	
Estimated relief (per cent)														
Total	80	90	80	75	65	80	40	90	85	90	85	85	50	
Stuffy nose	100	50	80	100	60	75	40	80	65	85	85	80		P
Rhinorrhea	100	85	80	75	50	75		85	80	95	90	85	50	O
Sneezing	50	85	80	75	60	100	50	90	100	95	85	80		O
Ocular	85	98	80	75	45	90		100	100	95		85+	50	R
Cough	75	100						90		90	80	90		
Wheezing		95						100		90+	100			
Malaise		85	80	90		90+		95	65		85	85	50	
Reactions														
Nasal	0	0	+	0	0	+	+	+	0	0	0	+	+	0
Bronchial	+	0	0	0	0		+	0	0	0	+	+	0	0
Antihistamines needed														
Early	++	++	++	++	++	++	++	++	++	++	++	++	++	++
Late (after maximal relief)	0	0	0	+	0	0	+	0	0	0	+	+	+	+
Compared with injection Rx‖	B		S	B				B		B	B	B	B	
Compared with no Rx‖	B	B	B	B	B	B	B	B	B	B	B	B	B	S

*Case 6, 1953. Had ineffectual preseasonal injections of pollen extract.
†Case 3, 1953.
‡Case 1, 1953.
§Had ineffectual coseasonal injections of pollen extract.
‖B, Better; S, same.

The over-all results were better in 1954 than in 1953, perhaps because more of the cases were simple hay fever and/or asthma with fewer complications than in several of the 1953 patients, or because smaller doses were used to start treatment.

DISCUSSION

The problems of local desensitization have been revived by Swineford[13, 14] and by Herxheimer.[12] They have shown that sensitized guinea pigs can be desensitized locally by aerosols of haptens[13] and whole antigens.[12] Herxheimer[8] doubts the validity of estimating the sensitivity of organs, such as the lungs, by testing a distant organ, such as the skin. The data of Schiller and Lowell[1, 5] support this doubt. Herxheimer[8] suggests further that local desensitization of a shock organ might be a more logical therapeutic approach than are the current methods of parenteral administration of allergens.

The 1953 observations described here had already been made when Herxheimer's[8, 10] reports of aerosol treatment of asthma and hay fever were found. The technique used here (consisting of a nebulizer, compressed air, and allergen extract) is much simpler and more suitable for routine clinical use than the closed circuit respirometer used by Herxheimer. The graduated increases in allergen aerosol concentration seem safer than the time increments of concentrated allergens used by him, in view of (1) the occasional fatal reaction in guinea pigs following a few seconds' exposure to hapten aerosols.[13, 14] (2) prompt asthma from a few whiffs of strong extract in Case 6, and (3) the occasional observation of severe asthma or shock from an injection of perhaps 0.1 ml. of 1:10,000 pollen extract, remembering that the injected extract is further diluted several thousand times by plasma before it reaches the bronchi.

The fall pollen season lasts from about August 19 to October 1 in central Virginia. The 1953 season was an average one. The 1954 season was hot and was the dryest in twenty-four years. It started a few days earlier, lasted about one week longer, and was more severe than the average.

The prime purpose of this study was to see if allergen aerosols would relieve fall pollen hay fever and asthma. They did. Numerous supplementary clinical experiments are suggested by these observations. Most of them could be done better in larger clinics than this.

The lack of controls is an obvious and basic omission. Certainly, the final role of specific aerosols in the treatment of inhalant allergy will not be assigned until extensive, controlled studies have been done. In spite of this omission, the background of this problem is sufficiently factual to lend validity to the observations reported here. Several examples will suffice to make this point: (1) the bronchi of guinea pigs are readily desensitized specifically by aerosols;[13, 14] (2) Herxheimer's results[6-12] were in agreement with those reported here, although the techniques were quite dissimilar; (3) Schiller and Lowell,[1-5] Herxheimer,[6-12] and others have demonstrated repeatedly the specificity of aerosols in the production of respiratory symptoms in sensitized animals and man. It has become almost axiomatic that antigens which produce specific inhalant allergic reactions can be used effectively in treatment.

Furthermore, it seems significant that, of the eight 1954 cases treated previously by pollen injections, seven reported more relief from aerosol therapy than from injections and one thought that the two methods provided about the same degree of relief.

Aerosol treatment with pollen extracts obviously is not the panacea for pollinosis. It is neither claimed nor implied that it should replace established specific methods of treatment.

SUMMARY

Twenty-one patients with fall hay fever, eight of whom also had asthma, were treated with aerosols of pollen extract.

The initial concentration of pollen extract varied from 1:40,000,000 to 1:8,000. The final concentration varied from 1:40,000 to 1:200. A part of the treatment was carried out at home by fifteen patients using a hand-pumped nebulizer.

Four patients received aerosol both pre- and coseasonally. Seventeen were given aerosol during the season only.

In 1953, six patients received aerosol plus pollen injections before and/or during the season. In 1954, only two patients received injection therapy.

In 1953, wheezing followed the aerosol in six cases, in four of these only when bronchitis was present. In 1954, six of the fourteen patients had nasal and four had bronchial reactions. All of these were mild and of short duration.

Severe asthma was precipitated once by an aerosol which was too concentrated. Transient asthma was precipitated in two patients who had not recognized asthma previously.

Aerosol therapy should be used with particular care in the presence of bronchitis.

In 1953, relief was estimated at 90 per cent in four cases, 80+ per cent in two, 50 per cent in one, 25 per cent in two, and no relief in two.

In 1954, eleven of fourteen cases reported relief estimated at 65 per cent or better. Relief began by the fourth day in all but one of the cases treated coseasonally by aerosol alone.

In contrast to Herxheimer's experiences, coseasonal therapy seemed effective and simple, and no evidence of "induced hypersensitivity" was encountered.

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ANAPHYLACTOGENIC PROPERTIES OF GELATIN AND ITS PRECURSORS

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ON OCCASION, questions have been raised relative to the allergenicity of edible gelatin. Some clinical reports have claimed its occurrence. Generally, however, gelatin has been considered as nonantigenic.

In addition, little attention has been given to the antigenicity of the parent products as distinct from the hydrolyzed gelatin. Traces of contaminants may elicit allergic symptoms, although gelatin itself may not be allergenic. It is for this reason that we included in this study the antigenic properties of bovine plasma, comminuted cooked ossein, and crude gelatin solution derived from the comminuted ossein, as well as food-grade gelatin and the most purified form—that used for intravenous injection.

MATERIALS

Bovine Plasma.†—In some pilot experiments, bovine serum and bovine plasma each reacted when used to challenge animals previously injected with comminuted ossein. Since the blood element reacting with the comminuted ossein was present in both the plasma and serum, bovine plasma was used exclusively in the experiments.

Comminuted Ossein‡.—

Stage A: This preparation is made from bovine bone demineralized with 5 per cent hydrochloride, washed with water, and then treated with saturated limewater solutions at 60° F. for approximately four weeks for removing extraneous materials such as albumins, mucins, and chondrins and also to initiate hydrolysis of collagen to gelatin. The limed stock is then washed with cold water and dilute acid to approximately pH 6.0 to remove lime salts. At this stage the ossein is ready to extract with hot water at approximately 160° F. This is the material which, in its entirety, formed the basis for the preparation of crude gelatin raw material.

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This is the seventh in a series of studies on the Allergenicity of Modified and Processed Foodstuffs.

The technical assistance of Kathryn M. Mayer, Joseph S. Thomas, and Dr. Mary Retsina is hereby acknowledged and deeply appreciated.

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†The bovine plasma and serum were generously supplied to us by Dr. M. S. Cooper, bacteriologist, Biological Production Section, Lederle Laboratories Division, American Cyanamid Company.

‡The comminuted ossein, crude, food-grade, and intravenous-grade gelatins were generously supplied to us by Dr. D. Tourtellotte, Director, Research Division, Charles B. Knox Gelatine Company, Inc. The gelatin solution contained 0.0108 gram protein nitrogen per cubic centimeter and the ossein 0.018 gram nitrogen per cubic centimeter.

Stage B: Two hundred thirty grams (40 per cent solids) of the stock was placed in 500 c.c. bottles with 290 grams distilled water added to fill, autoclaved at 15 pounds steam pressure for twenty minutes, then the cooked mix was poured into a Waring blendor and ground to a uniformly comminuted mush. This comminuted material was rebottled, sealed, and reautoclaved at 15 pounds steam pressure for twenty minutes.

Crude Gelatin.—From the extraction of the boiling just described, an amount of crude gelatin liquors was placed in sealed bottles and subjected to autoclaving at 15 pounds steam pressure for twenty minutes. This provided intermediate material, hot water extracted, but not filtered free of the traces of insoluble and particulate organic matrix.

Food-Grade Gelatin.—This preparation represents the finished commercial dry gelatin dissolved in distilled water, 6 per cent w/v with 0.9 per cent sodium chloride added to physiologic concentration. The commercial dry gelatin used in these experiments was that supplied by the Knox Gelatine Company and is known as Knox Sparkling Gelatine, U.S.P.

*Intravenous-Grade Gelatin.**—Since this product is designed entirely for parenteral use, more exacting selection is made of hard bone starting materials to avoid spongy bone, which can carry traces of hemoglobin and plasma protein residues which might become factors in allergenic responses to the finished product. More exacting care is exercised throughout the intravenous gelatin process to insure complete sterility at every stage. This is accomplished by working in completely enclosed equipment and at temperatures and reactions which are inhibitory to even air-borne contamination. In the case of the intravenous solution, all filtrations are made through special asbestos sterilizing pads, followed by immediate autoclaving in the final sealed flasks used for parenteral administration.

METHODS

The precipitin or complement fixation tests cannot be used because the gelatin congeals on cooling and because the comminuted ossein has particulate matter in it. We believe the induction of anaphylaxis in the guinea pig and the use of the Schultz-Dale technique on isolated tissue to be the most suitable for studies on the antigenicity of these materials.

Normal guinea pigs,† weighing approximately 250 grams, which had never been fed beef or gelatin, were used for the anaphylaxis studies.

Anaphylaxis was tested by the usual sensitization and shock procedures, with incubation periods of three to four weeks intervening. The sensitizing injections were made intraperitoneally and shock injections, unless otherwise specified, were given by the intravenous route. Whenever possible and when there was need for determining whether the absence of shock reaction was the result of absence of sensitization to a related material, the substance to which the animal was originally sensitized was employed as a second confirmatory

*Special gelatine solution, intravenous, Council of Pharmacy and Chemistry, J.A.M.A. 142: 32-33, Jan. 7, 1950.

†Supplied by Carworth Farms, New City, N. Y.

shock injection. A negative reaction obtained with a modified form does not justify the conclusion that allergenicity was not present unless proof is at hand that the animal had been sensitized in the first place. We believe this to be a sound method for determining the anaphylactogenicity of a modified protein.[1]

An adequate number of animals must be used in these experiments. By using ten or more animals, chance of missing a low sensitizing potential is reduced.

Because of the character of the comminuted ossein, the particulate bone elements made it impossible for us to use this material for intravenous shock injection.

The Schultz-Dale experiment was used to supplement graphically the anaphylaxis experiments.

EXPERIMENTS

Eleven animals were sensitized with comminuted ossein and challenged with bovine plasma. The comminuted ossein evidently contained some blood element derived from the homologous bovine plasma, as a cross anaphylactic reaction was demonstrated in this series. However, when the order was reversed and the animals were given a sensitizing injection of bovine plasma and challenged with the comminuted ossein, there was no demonstrable evidence of a cross reaction in eighteen animals tested. When a second injection was given, using bovine plasma to which the animals had been sensitized, seventeen of the eighteen animals died in anaphylactic shock. This proved them to be highly sensitive to the plasma and not to the ossein (Table I). This indicates that the bovine blood elements present in ossein were there in extremely small amounts because, although there was enough present to sensitize, there was not enough present to shock plasma-sensitized animals.

TABLE I

NUMBER OF ANIMALS	SENSITIZING INJECTION	SHOCK INJECTION	RESULTS		SECOND SHOCK INJECTION	RESULTS	
11	Comminuted ossein 2 c.c. Ip.	Bovine plasma 0.2 c.c. Iv.	2 animals 4 animals 5 animals	++++ + 0			
18	Bovine plasma 1 c.c. Ip.	Comminuted ossein 2 c.c. Ip.	0		Bovine plasma 0.3 c.c. Iv.	17 animals 1 animal	++++ 0

Key to symbols:
+, Moderate dyspnea.
++, Dyspnea and convulsions.
+++, Dyspnea, convulsions, collapse, and recovery.
++++, Anaphylactic death with typically ballooned lungs.
Ip., Intraperitoneal.
Iv., Intravenous.

Table II shows data on attempts to sensitize animals to ossein and to determine whether or not highly purified intravenous-grade gelatin retained any of the blood-contaminating antigenic elements found in the ossein.

There was no evidence of cross reaction with the gelatin in fourteen animals sensitized with comminuted ossein and challenged with intravenous-grade gel-

TABLE II

NUMBER OF ANIMALS	SENSITIZATION COMMINUTED OSSEIN	SHOCK IV.-GRADE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
14	2 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	1 animal ++++ 1 animal +++ 6 animals + 6 animals 0

atin. When these same animals were subsequently challenged with bovine plasma, eight proved anaphylactic, showing that the comminuted ossein contained some blood element, whereas the intravenous-grade gelatin was completely devoid of it.

The next grade of purification after the ossein is crude gelatin. Nineteen animals were injected with crude gelatin and challenged with bovine plasma. There was no evidence that the blood elements were present in the crude gelatin (Table III). When these nineteen animals were subsequently challenged with crude gelatin they were all negative.

TABLE III

NUMBER OF ANIMALS	SENSITIZATION CRUDE GELATIN	SHOCK BOVINE PLASMA	RESULT	CONTROL SHOCK CRUDE GELATIN	RESULT
19	3 c.c. Ip.	0.3 c.c. Iv.	0	0.3 c.c. Iv.	0

In the next series (Table IV), nineteen animals were sensitized to bovine plasma and challenged with crude gelatin with negative results. A second challenging dose of bovine plasma showed them to be highly sensitive to bovine plasma.

TABLE IV

NUMBER OF ANIMALS	SENSITIZATION BOVINE PLASMA 1 c.c. Ip.	SHOCK CRUDE GELATIN	RESULT	CONTROL SHOCK BOVINE PLASMA	RESULT
19		0.3 c.c. Iv.	0	0.3 c.c. Iv.	14 animals ++++ 2 animals + 3 animals 0

Food-grade gelatin was then tested for allergenicity (Table V). In the first twenty-six animals (Group I) food-grade gelatin was used as a sensitizing dose and bovine plasma as a challenging dose. There was evidence of the presence of bovine blood element in the food-grade gelatin in only two of these. In Group II, nineteen animals were injected and challenged with the same food-grade gelatin. There was no evidence of sensitization to the gelatin itself, although one showed the presence of some plasma element when the animals were given a second shock dose with bovine plasma.

In Group III, twenty-two animals were sensitized with bovine plasma and challenged with food-grade gelatin. In no instance was a blood element demonstrated in the food-grade gelatin when used as a challenging dose. However,

TABLE V

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	26	Food-grade gelatin 3 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	1 animal ++++ 1 animal + 24 animals 0		
II	19	Food-grade gelatin 3 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	1 animal ++++ 18 animals 0
III	22	Bovine plasma 1 c.c. Ip.	Food-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	20 animals ++++ 1 animal ++ 1 animal 0

when these twenty-two animals again were challenged with the bovine plasma with which they were originally sensitized, twenty-one demonstrated profound anaphylaxis.

In a total of forty-five animals, therefore, there were only three instances, or 6.6 per cent, where sensitization to the blood elements was demonstrated by the food-grade gelatin. In twenty-two animals, however, when the food-grade gelatin was used as a shock agent in animals exquisitely sensitive to bovine plasma, there was no evidence that the contaminating blood elements were present in sufficient amounts to produce a shock reaction.

Therefore, in none of a relatively large series of animals was food-grade gelatin shown to be anaphylactogenic to itself. However, there was evidence that there is an extremely slight amount of the blood element found in the food-grade gelatin which occasionally was able to sensitize but never was able to produce shock reactions.

TABLE VI

GROUP	NUMBER OF ANIMALS	SENSITIZATION	FIRST SHOCK	RESULT	SECOND SHOCK	RESULT
I	10	Iv.-grade gelatin 2 c.c. Ip. 5 days later, 1 c.c. Ip.	Bovine plasma 0.3 c.c. Iv.	0	Iv.-grade gelatin 0.3 c.c. Iv.	0
II	30	Bovine plasma 1 c.c. Ip.	Iv.-grade gelatin 0.3 c.c. Iv.	0	Bovine plasma 0.3 c.c. Iv.	19 animals ++++ 2 animals +++ 3 animals + 6 animals 0

The most purified form of gelatin is the one used for intravenous injection. In Table VI, Group I, there were ten animals given two sensitizing injections of intravenous-grade gelatin, five days apart, and challenged with bovine plasma. They were consistently negative. When challenged with intravenous-grade gelatin again, they were also proved to be negative, indicating that this gelatin was devoid of allergenicity. In Group II thirty animals were sensitized with bovine plasma and in no instance was there any evidence of cross reaction when challenged with intravenous-grade gelatin. The animals were sensitive to a high degree, for twenty-four of the thirty showed anaphylaxis when challenged with the original bovine plasma.

Schultz-Dale experiments, an example of which is shown in Fig. 1, graphically epitomize the studies of the antigenicity of gelatin. Virgin female guinea pigs sensitized to comminuted ossein were used in these experiments. The sensitized uterus was immersed in oxygenated Tyrode's solution. The uterine strip was challenged with food-grade gelatin (*A*). There was no evidence of reaction. After several minutes, the strip was challenged with bovine plasma (*B*). A very prompt maximal contraction ensued. The bath was changed with fresh Tyrode's solution (*C*). Bovine plasma was again placed in the bath (*D*). An immediate secondary contraction of lower amplitude ensued. The Tyrode's solution was again replaced (*E*). Bovine plasma was again placed in the bath (*F*). There appeared to be a refractoriness, followed several minutes later by a delayed reaction. The bath was changed again (*G*) and bovine plasma (*H*) was put in for the fourth time. The muscle showed complete refractoriness. Finally, without further change of bath, the muscle was challenged with histamine acid phosphate (*I*), which produced a maximal contraction.

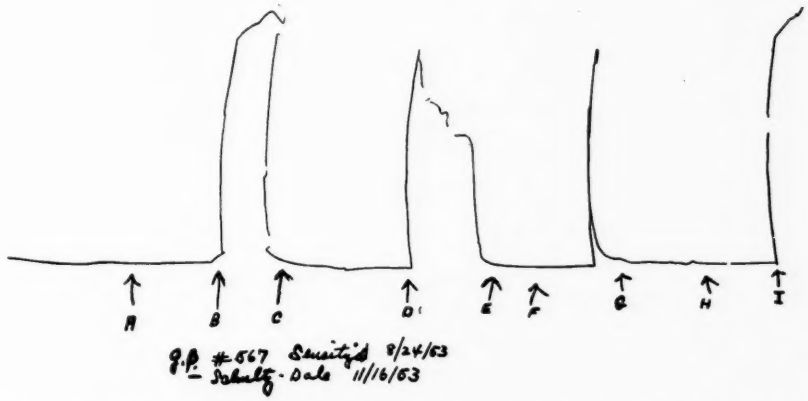

Fig. 1.—Animals sensitized with comminuted ossein and tested with food-grade gelatin and bovine plasma. *A*, Food-grade gelatin; *B*, bovine plasma; *C*, washed; *D*, bovine plasma; *E*, washed; *F*, bovine plasma showing refractoriness and a delayed reaction; *G*, washed; *H*, bovine plasma, desensitization; *I*, histamine acid phosphate.

This experiment shows in a vivid manner that when the precursor (comminuted ossein) of the food-grade gelatin is used as a sensitizing agent, there is no evidence of antigenicity when challenged with food-grade gelatin, but there is definite evidence of sensitization to some element in the blood plasma. The secondary contraction at *D* and the delayed contraction at *F*, when the uterine strip was challenged twice with bovine plasma, suggest that the first challenge dose did not result in a complete refractoriness. This might be due to a multiplicity of antigens in the bovine plasma. The final refractoriness indicates complete desensitization of all the fractions in the bovine plasma. The reaction to histamine proves the validity of the desensitization by showing that the uterine strip retains its viability and optimal functioning.

SUMMARY OF EXPERIMENTS

I. (See Table VII.) Comminuted ossein was found to sensitize against bovine plasma in fourteen of twenty-five animals (56 per cent). Food-grade gelatin, refined from comminuted ossein, sensitized only three of forty-five animals (6.6 per cent) when challenged with bovine plasma. In none of thirty-two animals did intravenous-grade gelatin sensitize against bovine plasma.

TABLE VII. COMPARATIVE REACTIVITY OF CONTAMINATING BOVINE BLOOD ELEMENTS IN CRUDE AND REFINED GELATINE WHEN USED AS A SENSITIZING AGENT

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULTS		
			POSITIVE	NEGATIVE	PER CENT POSITIVE
25	Comminuted ossein	Bovine plasma	14	11	56.0
45	Food-grade gelatin	Bovine plasma	3	42	6.6
32	Iv.-grade gelatin	Bovine plasma	0	32	0.0

II. (See Table VIII.) In none of 141 animals sensitized with bovine plasma, comminuted ossein, crude gelatin, food-grade gelatin, and intravenous-grade gelatin was there any reaction when the animals were challenged with any of the forms of unrefined or refined gelatin.

TABLE VIII. NONREACTIVITY OF CRUDE AND REFINED GELATIN WHEN USED AS ANAPHYLACTIC SHOCK AGENTS

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	RESULT
14	Comminuted ossein	Iv.-grade gelatin	0
9	Crude gelatin	Crude gelatin	0
19	Bovine plasma	Crude gelatin	0
19	Food-grade gelatin	Food-grade gelatin	0
22	Bovine plasma	Food-grade gelatin	0
30	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0
18	Bovine plasma	Comminuted ossein	0

III. (See Table IX.) Whether the intravenous-grade gelatin was used as a sensitizing injection or as a shock injection, in no instance in fifty-eight animals was there any sensitization or shock manifested, even when they were cross-tested with bovine plasma.

TABLE IX. ABSENCE OF ANY ANAPHYLACTOGENIC REACTIVITY OF INTRAVENOUS-GRADE GELATIN

NUMBER OF ANIMALS	SENSITIZATION	SHOCK	ANAPHYLAXIS
14	Comminuted ossein	Iv.-grade gelatin	0
24	Bovine plasma	Iv.-grade gelatin	0
10	Iv.-grade gelatin	Bovine plasma	0
10	Iv.-grade gelatin	Iv.-grade gelatin	0

COMMENT

Gelatin does not exist as a natural protein but is derived from hydrolysis of the insoluble connective tissue protein collagen which exhibits no demonstrable antigenic effects.

The nonantigenicity of gelatin was thought to be due to a deficiency of aromatic groupings of its amino acid constituents, tyrosine and tryptophane. There are several other possible explanations for the immunologic inertness of this protein. It is deficient in carbohydrate groupings. It may be excreted rapidly in the urine after intravenous injection into the animal. It is usually prepared from collagen by prolonged treatment with boiling water or steam and this treatment may well be sufficient to destroy any antigenic power of the preparation.

In explanation of the foregoing are several investigations[2] which showed that the introduction of aromatic groupings, including tyrosine, into gelatin does not produce a compound which has antigenic powers comparable with those of the majority of proteins.

Similarly, the failure does not appear to be due solely to lack of tyrosine even when carbohydrates are added.[3]

With reference to its rapid excretion via the kidneys, Haurowitz and his co-workers[4] believe that the major part of ingested gelatin or gelatin derivatives is so rapidly excreted in the urine that little remains in the body to induce antibody formation. Gelatin and several other proteins with molecular weights of less than 70,000 are rapidly excreted in the urine by anesthetized dogs.[5] Proteins such as egg albumin and Bence-Jones protein, both of which are excreted by the kidneys, are known to be fully antigenic. Loss by excretion in the urine, according to Wormall,[6] cannot be the only factor that determines the nonantigenicity of gelatin.

The last explanation (that is, the effect of prolonged heating in boiling water), we believe, has validity because it has been shown that albumin heated in the presence of water becomes coagulated and does have a definite reduction in antigenicity.[7]

The studies detailed in this article demonstrate that gelatin is a nonantigen and that any antigenicity found in its crude precursor (comminuted ossein) was due solely to contamination with bovine blood elements. In no instance did crude or refined gelatin serve as an anaphylactogen. The presence of contaminating blood elements was found in 56 per cent of the experiments with comminuted ossein and in only 6.6 per cent of the experiments with food-grade gelatin. The anaphylactogenicity of the blood elements present in the comminuted ossein and crude gelatin mixtures persisted despite the heat and hydrolytic treatments during the process of gelatin preparation.

In no instance was any contaminating blood element found in the highly purified gelatin used for intravenous blood replacement.

CONCLUSIONS

1. Within the limits of our experiments, we can state that gelatin is non-anaphylactogenic.

2. The antigenicity attributed to gelatin is related to a contamination by some blood elements of the species from which the gelatin is derived. This antigenic element is present in both the plasma and serum and apparently is highly resistant to heat.

3. The presence of blood contaminants in comminuted ossein was demonstrated in the majority (56 per cent) of tests on animals sensitized to this precursor of refined gelatin. It was found to a negligible degree (6.6 per cent) in sensitization tests with the food-grade gelatin. It was completely eliminated in the intravenous-grade gelatin (Knox).

4. The blood contaminant in the comminuted ossein and the food-grade gelatin was present in so minute an amount that it did not induce any anaphylactic reactions in animals sensitized to the whole plasma.

5. Because no authentic case of gelatin sensitization has been demonstrated in man, we were unable to obtain gelatin antibody-containing blood for human studies. We therefore must assume that because it is nonanaphylactogenic for the animal, it must be nonallergenic for man.

6. Any allergenicity in man that might be attributed to gelatin is due to contamination with blood elements of the species from which it is derived and not to antigenicity of the gelatin itself.

In recent studies, Maurer[8, 9] reports that he found normally occurring antibodies to gelatin in man. He also found them in herbivorous as well as carnivorous animals. This naturally occurring antibody is therefore not related to the diet of the animal. It may arise as a result of some relation between the collagen system and gelatin metabolism but this is uncertain. The antibody is not concerned in the production of the wheal reaction and apparently is not an anaphylactic antibody.

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ELECTROPHORETIC SEPARATION OF SKIN-SENSITIZING ANTIBODY FROM THE SERA OF RAGWEED-SENSITIVE PATIENTS*

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THE skin-sensitizing antibody has been found to be associated with many of the common forms of allergy. It differs from precipitating antibody in that it is more heat labile and does not produce a precipitate with its specific antigen in vitro. It is usually detected by means of skin testing procedures and it can be transferred by the Prausnitz-Küstner test, using whole serum. Precipitating antibodies appear to be present in the gamma globulin fraction of sera. On the other hand, the localization of skin-sensitizing antibody to any particular protein fraction of sera is not well established.

Scherrer,[1] using salting out methods with ammonium sulfate and sodium sulfate, was able to show that skin-sensitizing antibody in sera of patients sensitive to grasses, ragweed, and egg white was mainly present in the pseudo-globulin fraction, partly in the euglobulin fraction, and absent from the albumin fraction. Using a similar method, Stull, Sherman, and Cooke[2] demonstrated that the skin-sensitizing antibody in sera of patients sensitive to ragweed was associated with the pseudo-globulin fraction. Newell and associates[3] used the Tiselius electrophoresis apparatus to separate the sera of patients sensitive to ragweed and rabbit dander, and were able to show that sensitizing antibody was present in the gamma globulin fraction but not in the beta globulin or albumin fractions.

Using electrophoretic convection methods of studying allergic serum, Campbell and his associates[4, 5] have reported skin-sensitizing activity in the alpha and beta globulin fractions in one case. Cooke and associates[6] subjected allergic sera to electrophoresis and concluded from their experiments that the bulk of sensitizing antibody was not associated with gamma globulin. Menzel and associates,[7] using chemical and electrophoretic methods, studied the sera of ragweed-sensitive patients and found the skin-sensitizing antibody to be associated with the beta globulin and gamma globulin and possibly the alpha$_2$ fractions, but not with the alpha$_1$ or albumin fractions. Vaughan, Favour, and Jaffee[8] studied the skin-sensitizing antibody in ragweed hay fever patients, using Cohn's alcohol precipitation method (Method 10). The skin-sensitizing antibody was

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shown to be concentrated in the proteins of Fraction IIId, which was believed to consist primarily of beta globulins. Considerable activity was also noted in other fractions which contained almost no gamma globulin. In one serum containing reagins for ragweed pollen, Loveless and Cann[9] have found the activity to be distributed through the electrophoretic gamma and beta fractions. The study herein reported is an attempt to determine with which serum fraction the skin-sensitizing antibody is associated, using starch electrophoresis for serum fractionation.

METHOD

1. *Selection of Patients.*—Patients with hay fever or asthma, who demonstrated large positive reactions to various allergens by skin testing, were selected for this study. They were examined carefully to eliminate the possibility of previous infection with syphilis or virus hepatitis. A 30 ml. aliquot of fasting venous blood was taken under sterile conditions; the serum was withdrawn and stored at 2° C. until fractionation. A small quantity of the whole serum was passed through a Swinny-Seitz filter and a site was prepared in a nonallergic subject. Forty-eight hours later this site was tested with the appropriate antigen, and if a satisfactory passive transfer test was obtained the serum was fractionated.

2. *Methods of Separation.*—All sera were analyzed at 0.8° C. by free electrophoresis in Veronal buffer (pH 8.6, ionic strength 0.1[16]) in a Tiselius apparatus manufactured by Specialized Instruments Corporation, Belmont, California. Because of the higher optical sensitivity of this apparatus, the sera were diluted with buffer to 1 per cent protein concentration and dialyzed against the buffer for forty-eight hours prior to electrophoresis.

The relative concentrations of the various components were calculated from the schlieren patterns by the method of Tiselius and Kabat.[10] The total protein content of the sera was determined refractometrically and did not differ from normal values.[16]

For fractionation of sera into albumin and the various globulin fractions (alpha, beta, gamma), electrophoresis on supporting media was used. The findings of Kunkel and Slater,[11, 12] that potato starch molded into a rectangular block was an adequate medium for electrophoretic fractionation of complex protein mixtures, were confirmed and their method, slightly modified, was used throughout these experiments.

A trough of desired dimensions was made of a glass plate, and two 1.3 cm. Plexiglas bars were clamped to its sides. Thick wax paper was used for lining the trough. Filter paper pads were inserted at the ends of the trough and served also as bridges between the trough and buffer vessels. The potato starch* was thoroughly washed twice with distilled water and excess moisture was removed on a Buchner funnel. Finally buffer solution was used as a displacing agent, providing an additional wash and resulting in the saturation of the starch with buffer ions. Sufficient buffer was then added to the starch to make it into a

*Amend potato starch for chromatography was used in most of these experiments.

slurry. This was poured into the trough, excess moisture was removed by drying with filter papers, and finally the upper surface of the block was planed off with a wide spatula.

The serum to be separated was either injected lineally across the middle of the block or else mixed with some starch and poured into a rectangular hole cut out of the starch block. Good separation into straight and narrow bands was obtained when the width of the zone of application of the serum did not exceed 15 to 20 mm. and was uniform across the whole width of the starch block. This was best achieved with the second method of application. Application of the serum across the middle of the block prevented its displacement by chromatography and resulted in a sharpening of the zone of application during the two-hour equilibration with buffer which usually preceded electrophoresis. At the end of this period, the upper surface of the block was covered with a sheet of wax paper to minimize evaporation. The length of the block was 108 cm., its height was 1.3 cm., and the width varied from 5 to 10 cm., depending on the amount of serum applied. This varied between 2 and 6 ml.

The two buffer vessels filled to the same level were connected by filter paper wicks with the electrode vessels containing silver-silver chloride electrodes in 20 per cent potassium chloride solution. These electrodes were used in preference to the conventional platinum or carbon and steel electrodes, since no change in pH occurred. The buffer used was Veronal, at pH 8.6 and ionic strength 0.05. This ionic strength was used, rather than the usual ionic strength of 0.1, since it gave as good a resolution and allowed the application of higher voltages without undesirable increase in amperage. For good separation, the potential applied was about 1,000 volts and the current varied from 18 to 36 Ma., depending on the width of the starch block.

The electrophoretic separation was carried out in the cold room at 2 ± 1° C. The temperature measured with a thermometer inserted into the block did not exceed 14° C and was usually around 10° C. The length of the experiments varied between eighteen and twenty-four hours, and the separation of the albumin peak from the gamma globulin peak ranged from 30 to 45 cm. The migration of the albumin could be seen easily, due to the color of the various pigments associated with it. At the end of the experiment, the region over which the serum fractions were distributed was determined by applying a filter paper strip along the surface of the block. After removal it was stained with bromophenol blue. This "print" revealed distinct bands corresponding to the various protein components.

For elution of the serum fractions from the starch, the block was cut into segments 1 cm. wide. These were placed in sintered glass funnels and eluted by displacement with saline under suction. This process was hastened with suction, and foaming invariably occurred. In the later experiments, a speedier and more convenient method of elution was used. This consisted of transferring the starch segments into plastic tubes provided with perforated bottoms which were covered by filters, 1 to 2 mm. thick, made of paper powder. The tubes were suspended in 40 ml. standard centrifuge tubes (about 2.5 cm. from the bottom

of the latter) on three "fingers" protruding from the sides to the center of the centrifuge tubes. Centrifugation at about 2,500 r.p.m. for three minutes was sufficient for elution. Addition of saline solution to each plastic tube, followed by further centrifugation, insured complete elution of the protein from each starch segment. This modification reduced the time of elution of 70 to 80 tubes to about three hours, eliminated foaming, and prevented any starch particles from being carried into the eluate. It was found that contamination of the eluate with small amounts of starch interferred with the determination of protein concentration, and such contamination was avoided by this method of elution. The total volume of the eluate of each segment amounted to about 7 to 9 ml. An equal aliquot of 1.0 ml. from each tube was used for protein determination by the method of Lowry and his associates,[13] and a plot of relative protein concentration led to a clear delineation of the zones containing the electrophoretically separable serum fractions. The various protein fractions were reconstituted by pooling the eluates as indicated by the graph and concentrating by dialysis against 25 to 35 per cent dextran* solutions in standard Visking tubing. For comparison of the activity of the various fractions with that of untreated whole serum, all fractions were concentrated to the same volume as that originally used for electrophoretic separation. Lyophilization did not prove useful, as it resulted in concentrating, in addition to serum protein fractions, salts from the buffer and the saline to an undesirably high level. Concentration by dialysis against concentrated dextran solutions resulted in removal of both salts and water. To minimize the losses that occur in handling small volumes, concentration by dialysis was not carried beyond reducing the volume to about 5 or 6 ml. Final concentration to about 2 or 3 ml., when required, was achieved by partial lyophilization.

To determine whether the various chemicals used in the procedure caused skin irritation by themselves when injected into test subjects, two control fractions devoid of protein were prepared in each experiment. These were composed of the eluates from 10 to 15 cm. zones adjacent to the albumin and gamma globulin fractions and were concentrated by the same procedure.

In addition to characterizing the fractions from the curve obtained as described previously, each fraction was separately identified with its counterpart in the original serum by electrophoresis on filter paper. For this purpose the individual fractions were applied side by side with the untreated serum on a strip of filter paper (Whatman No. 1). After electrophoresis in a Veronal buffer of pH 8.6, ionic strength 0.1, in an apparatus similar to that described by Valmet and Svensson[14] and developed independently in this laboratory, the paper was dried and stained with amidoschwarz. The staining method used was that described by Pucar[15] and was preferred to that with bromophenol blue, since it gave much deeper and more easily discernible color to the various protein bands. All fractions were passed through the Swinny-Seitz filter, especially adapted for handling small volumes, into sterile vials.

*Supplied by courtesy of Dextran Corporation, Yonkers, N. Y. This batch contained dextrans with a molecular weight distribution of around 58,000.

3. *Detection of Activity.*—The skin-sensitizing activity of the serum fractions obtained from starch electrophoresis was determined by passive transfer. The subjects used for testing were medical students who had no history of allergic disease. They were skin tested to the common allergens before receiving the serum fractions, and only those with negative reactions were used. The back of the recipient was washed with alcohol, and 0.1 ml. of the whole serum and each of the serum fractions were injected intracutaneously in duplicate. The prepared sites were marked with methylene blue. Forty-eight hours later, the back of the recipient was again washed with alcohol and the sites were tested. Volumes of 0.01 ml. of the antigen solutions were injected into each of the prepared sites. One set of the duplicate sites was tested with the antigen to which the patient was sensitive and the other set of duplicate sites was tested with an antigen to which the patient showed no reaction on intradermal skin testing. The antigens were also injected into nonprepared areas of the recipient's back and the results in the prepared sites were compared with these control tests. The results were read twenty minutes after injection of the antigen. The extent and depth of the wheal, pseudopod formation, and extent and depth of flare response were evaluated and the results, after comparison with the control tests, were graded 0 to ++++.

RESULTS

A series of tests was carried out on the sera of patients with marked sensitivity to various allergens, as determined by skin testing. Preliminary testing by passive transfer using whole serum showed that in some of the cases skin-sensitizing activity in the sera was not present or was very small. These sera were discarded and only those showing relatively large Prausnitz-Küstner tests were used. It also became apparent that the selection of cases should be based on the results of skin testing by the intracutaneous method, rather than by the scratch method. It was noted that in one case testing the skin of the patient by the scratch method gave a large positive wheal, while the intracutaneous test was negative. This was probably a quantitative phenomenon, since the antigen used for scratch testing was many times more potent than that used for intracutaneous testing.

After separation, the whole sera and fractions were tested by the Prausnitz-Küstner technique in the back of a non-allergic recipient. The first serum studied showed positive reactions for skin-sensitizing antibody to ragweed pollen and will be described later in this article. The next four sera showed negative reactions for the sera fractions. These sera were from persons who were not ragweed-sensitive but were sensitive to a variety of other allergens, such as cat dander, grass pollens, and some foods. There seemed to be several possible explanations of the inability to demonstrate skin-sensitizing antibody in the serum fractions of these patients.

The possibility that the negative reactions obtained might be due to individual variations in the recipients was eliminated by repeating the tests, using several medical students for the same serum and its fractions. The results were

TABLE I. SERUM PROTEIN ANALYSES IN RAGWEED-SENSITIVE PATIENTS (ELECTROPHORETIC DISTRIBUTION IN PER CENT)

NAME	AGE	SEX	DIAGNOSIS	ALBUMIN	ALPHA$_1$ GLOBULIN	ALPHA$_2$ GLOBULIN	BETA GLOBULIN	GAMMA GLOBULIN	ALBUMIN GLOBULIN RATIO
P. D.	26	F	Extrinsic asthma-atopic dermatitis	51.97 53.10	6.06 6.29	11.08 8.82	12.40 13.15	18.46 18.63	1.08 1.13
D. B.	29	M	Hay fever	58.35	5.32	10.81	12.63	12.88	1.40
H. R.	29	M	Hay fever	56.55	5.68	9.15	14.71	13.90	1.30
G. L.	30	F	Hay fever	54.65	5.53	7.24	15.12	17.46	1.20
J. N.	27	M	Hay fever	51.97	4.49	10.90	16.41	16.23	1.08
D. C.	36	M	Hay fever	60.35	5.62	9.23	15.89	8.91	1.52
E. H.	38	F	Hay fever	58.73	5.56	8.73	17.68	9.29	1.42
R. J.	27	F	Hay fever	57.06	5.98	7.82	12.37	16.76	1.33
N. G.	48	M	Hay fever	59.39	6.17	8.23	14.93	11.28	1.46
G. C.	49	M	Hay fever	55.90	4.09	8.78	16.01	15.22	1.27
Average									
Normal men (30 cases) age 22 to 35				59.64± 2.99	4.67± 1.82	8.60± 1.43	12.92± 1.27	14.33± 2.45	1.48± 0.19
Normal women (14 cases) age 18 to 25				58.40± 2.31	5.17± 0.73	9.81± 0.98	12.42± 1.18	14.18± 1.76	1.40± 0.14

similar in all cases. It was known that serum stored at 2° C. retains its skin-sensitizing activity for many months, but the effect of aging upon serum fractions that are dissolved in buffer was not known. Subsequent tests with sera fractions which were positive showed that these fractions did lose activity, even when stored in the frozen state. In addition, the whole sera and sera fractions

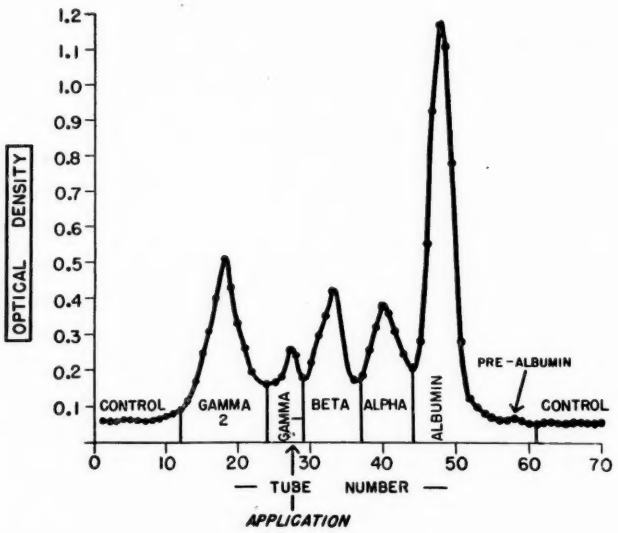

Fig. 1.—A protein distribution curve obtained by starch electrophoresis, showing the subdivision into various fractions.

were usually Seitz-filtered only once, but on occasion isolated fractions were filtered twice or more. It was found that the skin-sensitizing activity decreased markedly with each filtration. In view of these findings, it was decided to perform the fractionation procedure as rapidly as possible and to use the fractions

for Prausnitz-Küstner test as soon as they were obtained. Subsequent fractions were Seitz-filtered only once, immediately before they were used for passive transfer testing.

Since ragweed pollen appears to be one of the most antigenic of the common allergens and therefore might be expected to give rise to larger amounts of skin-sensitizing antibody in serum, it was decided that our attention would be directed, for the present, to sera of ragweed-sensitive patients.

The results of the free electropheresis of sera from patients sensitive to ragweed pollen are given in Table I, together with the average values obtained in this laboratory for thirty men and fourteen women. A study of this table reveals a trend for the gamma globulin and/or beta globulin fractions in ragweed-sensitive sera to be somewhat elevated as compared with our normal values.

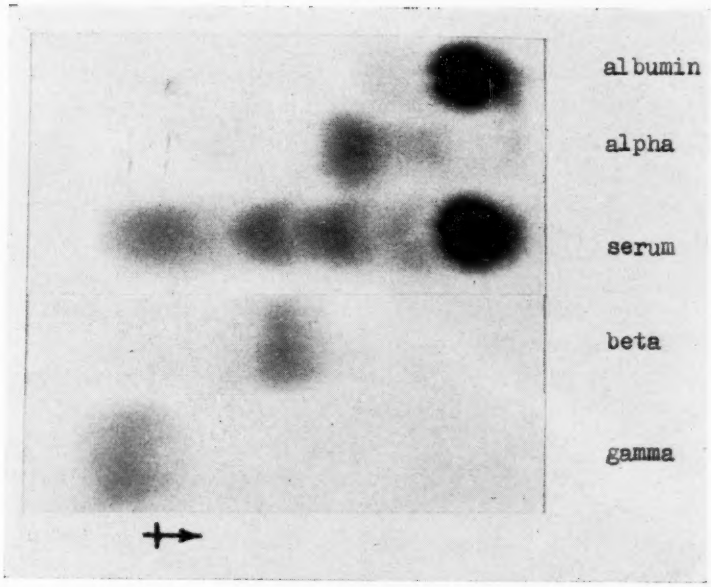

Fig. 2.—The characterization of the isolated serum fractions by paper electrophoresis.

A protein distribution curve obtained by starch electrophoresis and the subdivision into the various fractions is shown in Fig. 1. Due to a relatively large electro-osmotic effect, the gamma globulin fraction was found to move at pH 8.6 to the cathode. This confirms the findings of Kunkel and Slater.[11, 12] In the gamma globulin region, two peaks almost invariably were clearly delineated and are considered to be the two components, $gamma_1$ and $gamma_2$. This split of the gamma globulin fractions usually is not detectable in free electrophoresis.

The $alpha_1$ globulin peak usually was not resolved and, as revealed by electrophoresis on paper, was included with the albumin peak (Fig. 2). In most sera a small peak, usually absent in free electrophoresis, preceded the albumin on the starch (Fig. 1).

TABLE II. PASSIVE TRANSFER REACTIONS IN RAGWEED-SENSITIVE PATIENTS

NAME	AGE (YEARS)	SEX	DIAGNOSIS	INTRADERMAL SKIN TEST TO RAGWEED (1,000 PNU PER MILLILITER)	AGE OF SERUM AND FRACTIONS (DAYS)	PASSIVE TRANSFER TESTS TO RAGWEED (1,000 PNU PER MILLILITER)					
						WHOLE SERUM	WHOLE GLOBULINS	GAMMA GLOBULIN	BETA GLOBULIN	ALPHA GLOBULIN	ALBUMIN
P. D.*	26	F	Extrinsic asthma-atopic dermatitis	8+	110	++++		+++	+++	0	0
P. D.**	26	F			34	++++	++++	++++	++	0	0
D. B.*	29	M	Hay fever	++++	28	++++	++++	++	++	0	0
H. R.**	29	M	Hay fever	6+	28	++++		+++	++	0	0
G. L.**	30	F	Hay fever	5+	24	++++		++++	++	0	0
J. N.**	27	M	Hay fever	8+	25	++++		+++	++	0	0
D. C.*	36	M	Hay fever	++++	13	+++		+++	+++	0	0

*Gamma fractions free of beta.

**Gamma fractions containing some beta.

These sera and their fractions were tested for reaginic activity by passive transfer methods. Positive results were obtained in the fractions of seven sera from six ragweed-sensitive persons. Serum from one patient was obtained on two occasions with an interval of six months. Both sera and their fractions showed reproducible positive results. In four cases reaginic antibody was confirmed in the whole serum, but activity could not be demonstrated in the serum fractions. The seven positive results are shown in Table II. It can be seen that, in all cases, activity was demonstrable in the gamma and beta globulin fractions, but not in the alpha globulin and albumin fractions. In two cases, all the globulin fractions from the starch block were pooled together and, when tested, showed the same degree of whealing and erythema as the untreated whole sera. The control site prepared with fractions that did not contain protein gave negative reactions, as did all the sites tested with antigens to which the patients were not sensitive. While the techniques used in this study do not lend themselves to a quantitative interpretation of the data, it was noted that in four of the seven cases there was more activity in the gamma globulin than in the beta globulin fraction.

Subsequent to the Prausnitz-Küstner tests, these seven positive sera and their fractions were re-characterized as to their electrophoretic mobility and purity by paper electrophoresis (Fig. 2). The albumin and alpha globulin fractions were shown in all cases to be free of other serum protein components. The beta globulin fractions of the seven sera did not contain any detectable gamma globulin, but four of them contained traces of some faster-moving material with a mobility between that of alpha and beta globulins. The gamma globulin fractions of three of the seven sera did not reveal the presence of any other globulin fractions; the other four gamma globulin fractions showed traces of material moving with the mobility close to that of beta globulin.

It is obvious from these results that there was no skin-sensitizing activity associated with the albumin and alpha globulin fractions, that there was definitely activity in all beta globulin fractions, and that the activity in the slowest-moving component was almost certainly associated with gamma globulin.

DISCUSSION

The methods for serum fractionation used in previous studies of this nature have certain disadvantages. Separation by salting-out methods has been shown to result in fractions which contained more than one electrophoretically separable protein component.[3, 17] Alcohol fractionation methods suffer from the same disadvantage. The Tiselius electrophoresis method enables one to recover the fastest and slowest moving components in a relatively pure state. However, intermediate components are separable only with great difficulty and the amounts recoverable by this method are very small. The finding of previous workers using this method,[3, 18] that skin-sensitizing activity was associated with gamma globulin, is considered to be valid but conclusions regarding the activity of other fractions are open to question. The electrophoresis-convection method, although

a powerful tool for the separation of large quantities of material, produces fractions containing more than one electrophoretically separable component. Furthermore, it necessitates a relatively long period for the complete processing of serum.

Starch electrophoresis yields reproducible fractionations when used on the same serum, and the relative protein concentrations of the various fractions are of the same order of magnitude as those calculated from the schlieren patterns of free electrophoresis. The advantages of starch electrophoresis methods are rapidity of the separation, simultaneous separation of the sera into their electrophoretically separable components, and the possibility of direct characterization of the various components. Furthermore, in common with all electrophoretic techniques, it is one of the mildest methods of serum fractionation.

The results presented in Table I do not allow any conclusive generalization regarding the protein distribution in sera of ragweed-sensitive patients except that the albumin-globulin ratio shows a trend toward lower values than those of normal persons. Similar results in infective asthma were reported by Cooke and his co-workers.[6]

The passive transfer experiments demonstrate that skin-sensitizing activity in the sera of ragweed-sensitive patients is associated with beta and gamma globulin fractions and is not associated with albumin and alpha globulin fractions. The results of the characterization of the individual fractions by paper electrophoresis show conclusively that the albumin and alpha globulin components do not contain any other slower-moving components. Some of the beta globulin fractions contain traces of a component moving with a mobility close to that of $alpha_2$ globulin, but do not contain any slower-moving components. Thus, the activity found in the beta globulin fraction cannot be due to any other serum protein component. Three of the gamma globulin fractions showed no trace of other serum proteins, and the skin-sensitizing activity of these fractions cannot be attributed to faster-moving components. In the other four cases, traces of material with a mobility approaching that of beta globulin were found in the gamma globulin fraction. However, the high degree of activity found in these fractions, together with the unequivocal findings in the aforementioned three instances, is considered to be strong evidence that the skin-sensitizing activity in these fractions was in fact associated with gamma globulin. Further experiments, designed to eliminate interzonal fractions, are in progress and it is hoped that they will clarify this matter further. Since this work was completed, further studies have indicated that activity probably resides in the $gamma_1$ fraction primarily. This would explain the finding of activity in both beta and gamma fractions in the present work. In four cases, skin-sensitizing activity, while demonstrable in the whole sera of ragweed-sensitive patients, could not be elicited in the serum fractions obtained by starch fractionation. It is not apparent from our data why this activity could not be demonstrated after fractionation.

SUMMARY

1. The separation of ten sera from ragweed-sensitive patients into their electrophoretically separable protein components, using starch electrophoresis, is described. The advantages of the method are discussed and several modifications are presented.

2. The resulting sera fractions were tested by passive transfer techniques to determine the localization of skin-sensitizing antibody present in the whole sera.

3. It was found that the albumin and alpha globulin fractions have no skin-sensitizing activity, while both beta and gamma globulin fractions demonstrate significant skin-sensitizing activity. These findings and the results of previous work of this nature have been discussed.

We wish to acknowledge the skillful assistance of Mr. P. L. Rojowski and to thank Mrs. A. Elovic and Mr. E. de Vries for their help in this study.

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THE EFFECT OF CORTICOTROPIN AND CORTISONE ON RESPIRATORY FUNCTION IN BRONCHIAL ASTHMA

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Montreal, Quebec

HURTADO and Kaltreider[1] demonstrated some years ago that there is a consistent change in the pulmonary function of patients with bronchial asthma. In a group of five asthmatic patients they were able to show that the vital capacity was decreased, the residual capacity was increased, and the mid-capacity was elevated. These adverse changes were noted to improve following administration of epinephrine. Using improved techniques, Baldwin and others[2] confirmed these findings and showed in addition that the maximum breathing capacity was impaired in this disease. It was noted that the defects observed in the pulmonary function in bronchial asthma are qualitatively similar to those found in pulmonary emphysema. This naturally led to the problem of whether the defect in bronchial asthma could be due to pulmonary emphysema and whether emphysema could be a sequel of long-standing asthma.

Lukas[3] studied the pulmonary function in children with bronchial asthma, reasoning that secondary changes in the lung due to emphysema might be expected to be minimal in such a group. He was able to demonstrate that, at times when the patients considered themselves to be free of symptoms, their pulmonary function was comparable to that found in emphysema. There was a reduction in vital capacity and maximum breathing capacity, and an increase in residual capacity, with defective intrapulmonary mixing of gases. These changes could be reversed to some degree with bronchodilators. Similar results were found by Beale, Fowler, and Comroe[4] in an older group of asthmatic patients. Many of their patients demonstrated grossly abnormal findings, despite freedom from asthma for periods up to a year. An aerosolized bronchodilator was administered to some of the asthmatic patients, with a subsequent improvement in the abnormalities observed. This was interpreted as indicating that many asthmatic patients have bronchospasm in the absence of signs or symptoms.

Our interest in this problem arose during early studies of the effects of corticotropin (ACTH) in asthma.[5] Observations of the changes in pulmonary function were made at random and demonstrated that bronchospasm could be markedly relieved. However, detailed studies of the pulmonary function were not done at this time. It was decided to attempt a long-term study to evaluate

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the degree to which the emphysematous-like state in chronic asthma might be reversed, and to determine what role subclinical bronchospasm might play in the persistence of abnormal pulmonary function. The more complete and continuous relief afforded by corticotropin (ACTH) and cortisone[6, 7] in bronchial asthma seemed particularly suited to this problem and it was decided to use these hormones to eliminate the bronchospasm.

METHODS

Eight patients with bronchial asthma were selected at random and were hospitalized for two weeks at the beginning of the study. During this period, a complete medical and allergic work-up was done, including skin tests, hemogram, roentgenograms of the chest and sinuses, excretion of urinary steroids, and pulmonary function tests. After a control period, during which treatment was limited to providing symptomatic relief, the patients were started on corticotropin (ACTH) therapy for a further hospital period. They were then discharged on oral cortisone, returning at frequent intervals for repeat pulmonary function tests.

The pulmonary function tests employed included vital capacity and its subdivisions, maximum breathing capacity, residual volume, and the index of intrapulmonary mixing (I.I.M.). A 9-liter Collins respirometer was used to determine the vital capacity and the maximum breathing capacity. The residual volume was obtained from the functional residual volume by the nitrogen dilution method of Darling, Cournand, and Richards.[8] The index of intrapulmonary mixing, which is the percentage of nitrogen in an alveolar air sample after breathing oxygen for seven minutes, was also obtained by this method. Duplicate determinations of the functional residual volume were made and results were not accepted if there was a difference in excess of 3 per cent of the mean values. These results were compared with predicted normal values for each patient.[9, 10]

The results of this study are shown as individual charts of the response of each patient to therapy. The figures show the daily status of the patient. The treatment is indicated at the bottom of the charts and the symptoms, graded from 0 to 4+, as a line diagram. The maximum breathing capacity is shown by a line diagram, with the predicted normal value running across the chart at the appropriate level. The index of intrapulmonary mixing and residual volume/total capacity ratio are represented by figures, with the normal values at the left side of the chart. The vital capacity and its subdivisions are shown as blocks situated above and below a line representing the mid-capacity. The residual volume and total capacity are shown above the vital capacity. Normal values for lung volumes are represented at the left of the chart. The abbreviations used in the figures are as follows: I.I.M., index of intrapulmonary mixing; R.V./T.C. $\times$ 100, residual volume/total capacity ratio; E.R.V., expiratory reserve volume; I.C., inspiratory capacity; V.C., vital capacity; T.V., tidal volume; R.V., residual volume; T.C., total capacity.

RESULTS

Case 1.—The patient was a 19-year-old boy who developed asthma at the age of 6 years. His symptoms, which were confined to severe attacks of wheezing, occurred only in the summer until two years before this study, when they became perennial and increased in severity. During this period he gradually become more incapacitated, lost weight, and spent much of his time in bed. His chest,

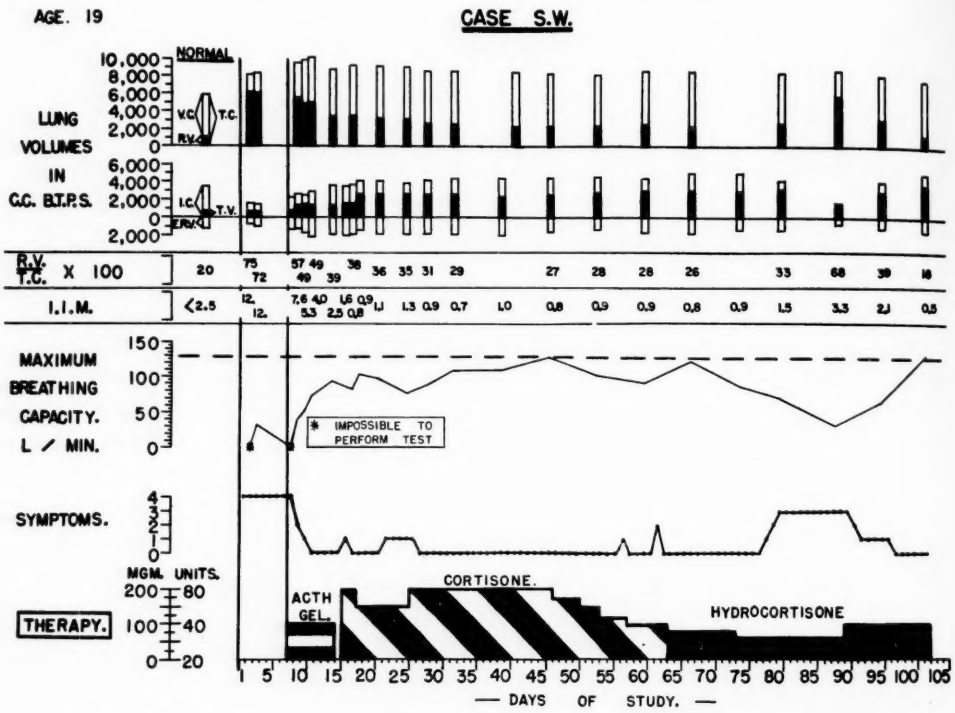


Fig. 1.—The effect of corticotropin, cortisone, and hydrocortisone therapy on the pulmonary function in a 19-year-old boy with extrinsic bronchial asthma. The daily status of the patient is shown. Treatment is indicated at the bottom, and symptoms immediately above. The maximum breathing capacity is shown as a line diagram and the predicted normal value as a broken line across the chart. The index of intrapulmonary mixing and the residual volume/total capacity ratio are represented by figures, with the normal values at the left. The vital capacity and its subdivisions are shown as blocks situated above and below a line representing the mid-capacity. The residual volume and total capacity are shown at the top of the figure. Normal values for the lung volumes are shown at the left of the figure.

I.I.M., index of intrapulmonary mixing; *R.V./T.C. × 100*, residual volume/total capacity ratio; *E.R.V.*, expiratory reserve volume; *I.C.*, inspiratory capacity; *V.C.*, vital capacity; *T.V.*, tidal volume; *R.V.*, residual volume; *T.C.*, total capacity.

on examination, showed a barrel-shaped appearance with considerable dorsal kyphosis. There were high-pitched rhonchi throughout the lung fields. Skin tests showed significant reactions to several inhalants and foods. He had not responded previously to conventional treatment and was thought to have resistant extrinsic asthma.

In Fig. 1 are shown the results of the pulmonary function tests. During the seven-day control period the symptoms were severe, and bronchodilators provided moderate relief for only a few minutes at a time. The pulmonary func-

tion tests were markedly impaired, as reflected by a great reduction in the vital capacity, a considerable increase in the total capacity and residual volume, and a very small maximum breathing capacity. There was a very high index of intrapulmonary mixing (12; normal, <2.5) and the residual volume/total capacity ratio (R.V./T.C. ratio) was greatly elevated.

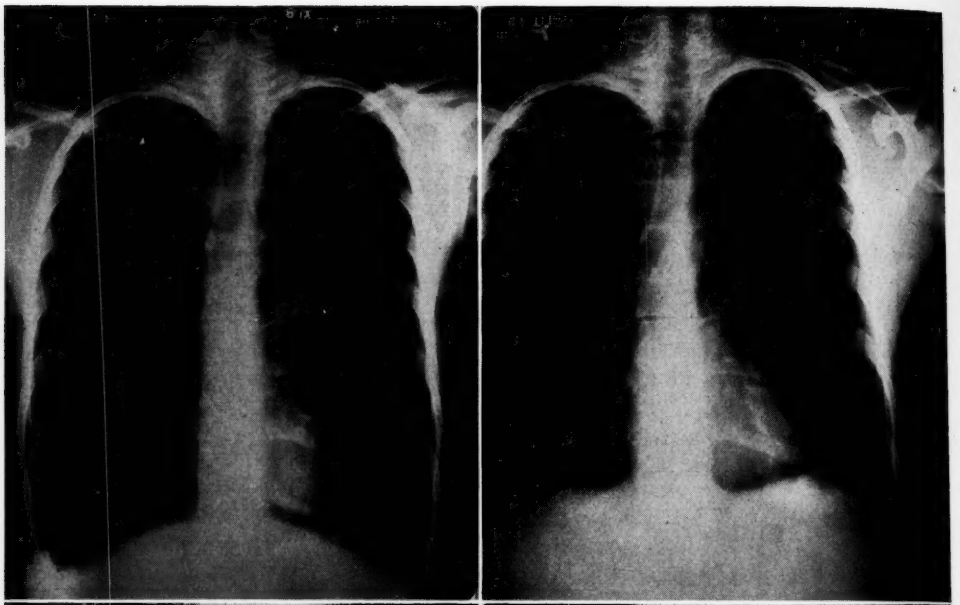
Following the administration of corticotropin (ACTH), 40 units subcutaneously of a long-acting preparation daily, asthmatic symptoms cleared rapidly and disappeared completely by the fourth treatment day. During this period the vital capacity improved rapidly, the residual volume diminished, the total capacity increased, and the maximum breathing capacity improved. A fall was seen in the I.I.M. from 12 to 2.5 and the RV./T.C. ratio reflected an improvement in the pulmonary function. The patient was discharged from hospital after seven days of corticotropin therapy and was followed as an outpatient while receiving oral cortisone. At first he required 200 mg. of cortisone daily to maintain freedom from symptoms, but later his requirements diminished. The vital capacity increased rapidly during the first two weeks and then showed a more gradual rise during the next two months. The residual volume showed a gradual fall over this period, but did not reach relatively normal values until fourteen weeks after beginning therapy. The total capacity, after an initial rise, also decreased slightly over the treatment period. A sharp initial improvement in the maximum breathing capacity was followed by a gradual increase to normal levels in the succeeding weeks. The index of intrapulmonary mixing reached normal values about a week after the start of therapy and continued to fall to values of less than 1. The R.V./T.C. ratio paralleled the fall in residual volume.

An unusual finding in this patient was the change seen in the tidal volume and respiratory rate following relief of his asthma. It was noted that the tidal volume gradually rose until it reached a value of 3,900 c.c., with a respiratory rate of 3 to 5 per minute. This finding persisted throughout the period of therapy and was apparently present at all times. This change was not seen in the other patients studied. During the treatment period oral cortisone was replaced by oral hydrocortisone. It became apparent that 60 mg. of hydrocortisone was insufficient to maintain a symptom-free state and the dose was increased. During this time, however, as will be seen in Fig. 1, symptoms of asthma returned and the pulmonary function tests once again showed abnormal values. The residual volume, in fact, rose to its original level. However, symptomatic improvement occurred on increasing the daily maintenance dose to 100 mg. of hydrocortisone and the pulmonary function tests returned to normal within a much shorter period of time.

Coincident with the changes just outlined, there occurred a pronounced change in the patient's physical status. His appetite returned, he gained weight, and returned to normal activities for a young man of his age. There was a marked sense of well-being, and for the first time the patient was able to obtain a job and plan for the future. The "emphysematous" appearance of the chest

Fig. 2.

A. *B.*



A. *B.*

Fig. 3.

Fig. 2.—Posteroanterior roentgenograms of the chest before (*A*) and after (*B*) five weeks of corticotropin and cortisone therapy in a 19-year-old male patient with extrinsic bronchial asthma.

Fig. 3.—Right lateral roentgenograms of the chest before (*A*) and after (*B*) five weeks of corticotropin and cortisone therapy in a 19-year-old male patient with extrinsic bronchial asthma.

seemed less marked, the dorsal kyphosis of his spine was reduced, and expiratory rhonchi were not present. The x-ray pictures of his chest before therapy and during the fifth week of therapy are presented in Figs. 2 and 3.

Case 2.—The patient was a 42-year-old woman with a history of asthma and generalized atopic dermatitis since the age of 18. At first her symptoms were worse in the summer and early fall. For the past several years, however, she has had perennial asthma exacerbated by infection. This was accompanied by cough and sputum at all times. Physical examination at the beginning of this study showed extensive atopic dermatitis in a small, undernourished woman. Her chest showed indrawing of the lower costal areas on inspiration with some increase of AP diameter of the chest. There were diffuse expiratory rhonchi over both lungs. An x-ray picture of the chest suggested early emphysema. Skin tests showed positive reactions to many foods and inhalants, but she always presented a difficult problem in spite of conventional therapy.

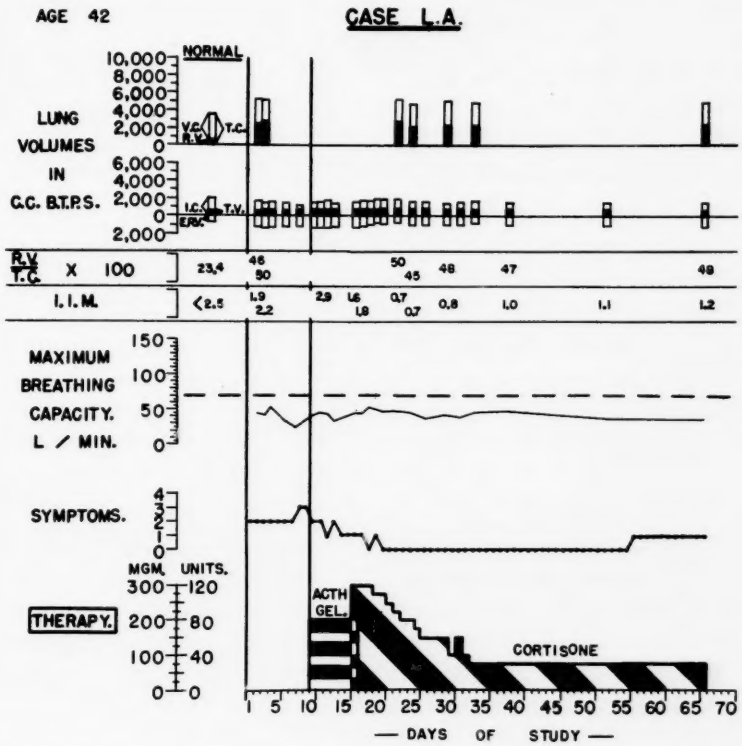


Fig. 4.—The effect of corticotropin and cortisone therapy on the pulmonary function of a 42-year-old woman with bronchial asthma. For explanation of symbols, see Fig. 1.

In Fig. 4 are shown the results of the pulmonary function tests in this case. As will be seen, during the control period there was more or less continual asthma of a moderate degree, which responded only slightly to routine bronchodilators. The vital capacity was not reduced, although the relation between the inspiratory capacity and the expiratory reserve volume indicated

that the mid-capacity had risen. The residual volume, however, was much increased (2,416 c.c., normal, 828 c.c.), as was the total capacity, and the R.V./T.C. ratio conformed with these findings (46; predicted normal, 23.4). The maximum breathing capacity was somewhat reduced, but the index of intrapulmonary mixing was within normal limits.

On the tenth hospital day, administration of 80 units daily of a long-acting preparation of corticotropin (ACTH) was begun and continued for six days. The patient was then given 300 mg. of oral cortisone daily and discharged. In response to this regime, the symptoms decreased slowly and the patient became symptom-free on the eleventh day of therapy. She remained free of clinical asthma for a period of one month, during which the cortisone was reduced to 75 mg. a day in divided doses, and then developed slight attacks at night and in the morning. The vital capacity did not change significantly in volume during the period of study, but it was noticed that the mid-capacity fell. The total capacity fell slightly during the eight weeks of treatment, as did the residual volume, but the ratio between them was not appreciably altered. The maximum breathing capacity did not change. The index of intrapulmonary mixing remained within normal limits. Thus, despite marked subjective improvement and freedom from asthma for a considerable period of time, no significant changes were seen in the pulmonary function tests.

Case 3.—A 30-year-old male patient had had asthma since the age of 2 years. His symptoms were continuous from late summer to November each year, with occasional attacks during the rest of the year. During the previous year he had more difficulty than formerly, with frequent chest colds which aggravated his symptoms. Physical examination was essentially normal with the exception of the chest, which presented a somewhat emphysematous appearance, with loud expiratory rhonchi heard throughout the lung fields. The x-ray picture suggested a fairly marked degree of emphysema. Skin tests showed many large reactions to inhalants; the foods were negative, but the patient avoided many foods because of suspected association with asthmatic attacks. His was considered to be a case of resistant extrinsic asthma.

The results of the pulmonary function tests are shown in Fig. 5. It will be seen that, while the asthma was moderate at the beginning of the ten-day control period, the symptoms abated to some extent without therapy. During this time the vital capacity, which was initially reduced, increased; the total capacity also increased and the residual volume remained elevated above normal. The R.V./T.C. ratio was also elevated (64; normal, 20), but showed some decrease on the tenth day due to a slight decrease in residual volume and an increase in total volume. The index of intrapulmonary mixing was elevated, but fell to near normal values by the end of the control period. The maximum breathing capacity rose during the control period, but was still substantially below the predicted normal.

Forty units of long-acting corticotropin (ACTH) daily was instituted on the eleventh hospital day and continued for four days. The patient was then dis-

charged on 200 mg. of oral cortisone daily. It will be noticed that the symptoms returned to some degree coincident with discharge from the hospital and, despite relatively large doses of cortisone (200 mg. daily), were not fully controlled. Still larger doses of cortisone (300 mg. daily) suppressed the symptoms and they did not return again, despite a gradual decrease in cortisone, during the period of observation. Under the influence of this regime, the vital capacity increased slightly and attained relatively normal values by the eighth day of treatment. The residual volume decreased gradually, as did the total capacity.

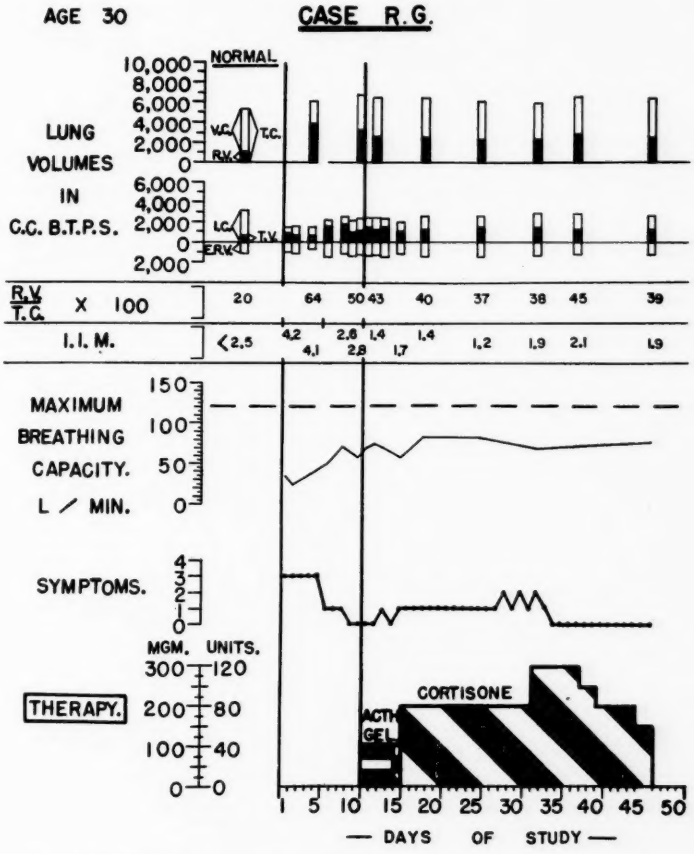


Fig. 5.—The effect of corticotropin and cortisone therapy on the pulmonary function of a 30-year-old man with extrinsic bronchial asthma. For explanation of symbols, see Fig. 1.

Both showed a slight rise, again coincident with an increase of symptoms during the third week of treatment, and then fell when the asthma was relieved. However, the residual volume, despite a substantial decrease, did not return to normal. The R.V./T.C. ratio showed a fall which paralleled that of the residual volume. There was a further slight rise in the maximum breathing capacity but it, too, did not return to normal. The index of intrapulmonary mixing

returned to normal values early in the treatment period. Again there was marked clinical improvement and freedom from asthma in the latter period of therapy.

Case 4.—The patient, a 29-year-old man, had had bouts of "bronchitis" each winter since the age of 1 year, which was diagnosed as asthma when he was 18 years of age. For the two years prior to his admission his attacks became more severe and prolonged, and each winter he had continuous trouble. Physical examination at the beginning of the study was relatively normal, with the

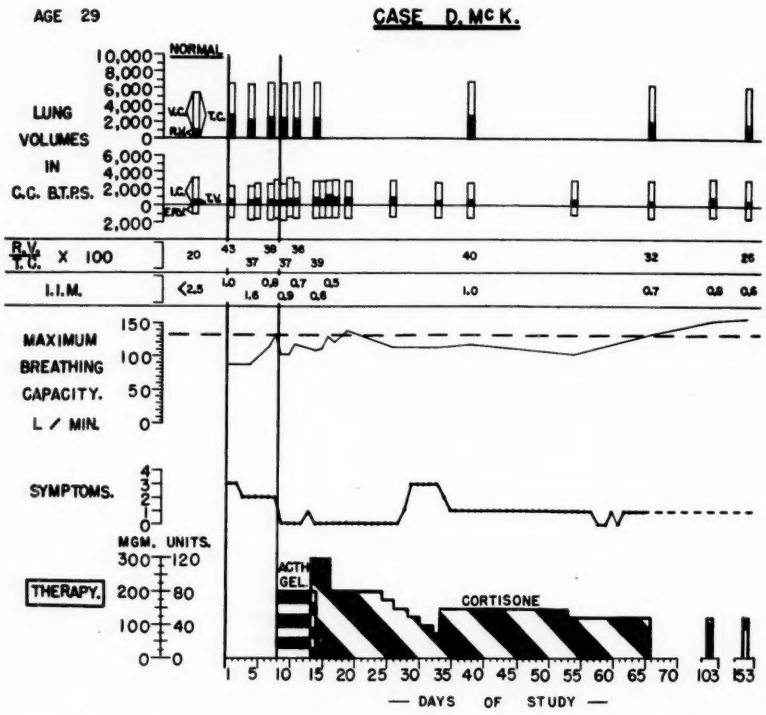


Fig. 6.—The effect of corticotropin and cortisone therapy on the pulmonary function of a 29-year-old man with extrinsic bronchial asthma. For explanation of symbols, see Fig. 1.

exception of the findings in the chest. There was some kyphosis and an increase in the AP diameter of the chest. Expiratory rhonchi were heard generally throughout the lung fields. X-ray examination of the chest was essentially normal, although the suggestion of early emphysema was made. Skin tests showed marked sensitivity to many inhalants and foods, and the patient was considered to have resistant extrinsic asthma.

Fig. 6 shows the changes in pulmonary function which occurred in this patient during the period of observation. At the beginning of the study there were moderately severe symptoms which decreased slightly during the seven-day control period. The vital capacity, which was a little reduced from the normal, increased slightly but remained impaired. The residual volume and

the total capacity were increased above the predicted normal and did not change. The R.V./T.C. ratio showed a similar trend. There was an increase in the maximum breathing capacity during the control period until it was relatively normal. The index of intrapulmonary mixing was within normal limits.

On the ninth hospital day, long-acting corticotropin (ACTH) therapy (80 units daily) was begun because of the abnormal pulmonary function values. After six days the corticotropin was withdrawn and the patient was discharged on a dose of 300 mg. oral cortisone per day. It will be seen that the symptoms disappeared on corticotropin therapy but reappeared when the cortisone dosage was lowered below 150 mg. daily. When the dose of cortisone was again increased to 150 mg. per day, the symptoms abated but did not completely disappear. More complete relief probably could have been obtained with a further increase in the dose but, due to the development of minimal side effects, it was decided not to increase the dose.

Under the influence of this therapy, the vital capacity increased a little and the mid-capacity fell, as indicated by the decrease in expiratory reserve volume and increase in the inspiratory capacity. The residual volume and total capacity did not change particularly until the second month of therapy, when they fell considerably. The R.V./T.C. ratio paralleled these changes, falling from 43 to 26. The values for residual volume at the end of the period of study were still slightly above normal. The maximum breathing capacity rose still further under the influence of therapy. The index of intrapulmonary mixing, which was normal at the beginning of the study, fell to very low normal values. There was a marked improvement in the clinical status of the patient during the period of study.

Case 5.—A 19-year-old female patient developed asthma at the age of 12 years. Her symptoms were perennial and particularly troublesome at night. For the fifteen days prior to this study, the patient received cortisone and hydrocortisone by mouth and her symptoms during this period were minimal. Physical examination revealed some increase of AP diameter of the chest but there were no rhonchi to be heard over the lung fields. The x-ray picture suggested a moderate degree of pulmonary emphysema. Skin tests were negative, and her asthma was believed to be primarily intrinsic in nature.

The results of the study on this patient are shown in Fig. 7.

Due to the previous cortisone and hydrocortisone therapy, which was discontinued on admission to hospital, the patient was relatively free of symptoms and had only minor attacks during the fifteen-day control period. The vital capacity was slightly reduced and it was noted that the mid-capacity began to rise toward the end of this period. The residual volume and total capacity were markedly elevated, despite the course of therapy immediately prior to this study. The R.V./T.C. ratio, which was elevated initially, fell from 53 to 40 during the control period, chiefly due to a rise in the total capacity. The maximum breathing capacity was depressed, but at no time during this study were the results considered valid due to the inability of this patient to perform this test satisfactorily. The index of intrapulmonary mixing was very low initially and rose gradually during the period of observation.

On the sixteenth hospital day, administration of 80 units of long-acting corticotropin daily was begun in order to see if it would influence the pulmonary function tests. After nine days of ACTH the patient was discharged on 100 mg. of oral cortisone per day. While receiving this therapy the patient continued to be relatively symptom-free except for occasional attacks. It will be noted that cessation of cortisone therapy at one period resulted in an immediate flare-up. During the treatment period the vital capacity increased and the mid-capacity was noted to fall again. The residual volume and the total capacity

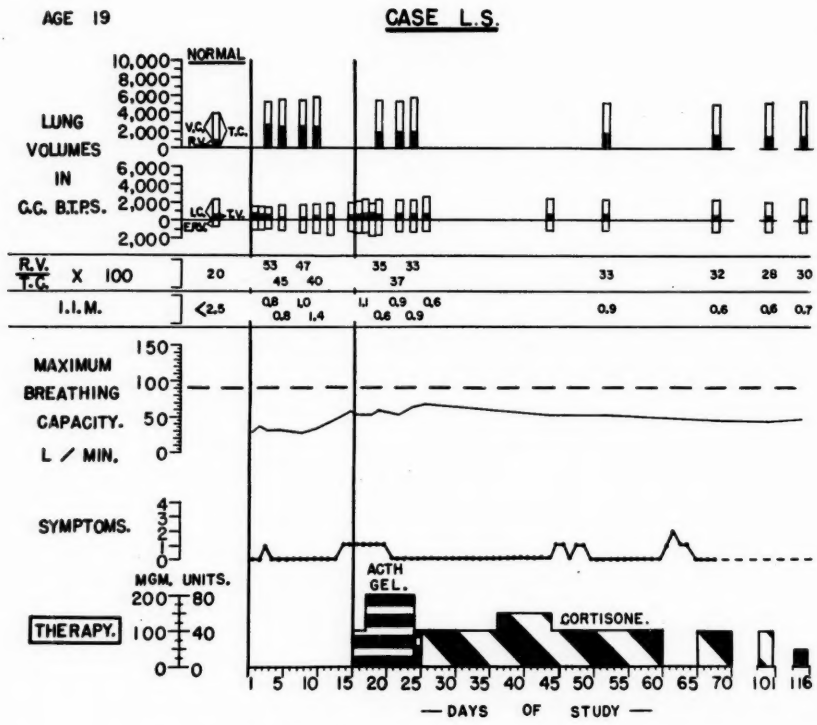


Fig. 7.—The effect of corticotropin and cortisone therapy on the pulmonary function of a 19-year-old girl with intrinsic bronchial asthma. For explanation of symbols, see Fig. 1.

decreased, although during the period of study the residual volume did not return to normal. The R.V./T.C. ratio paralleled these findings. The index of intrapulmonary mixing fell again to quite low levels. The patient remained well except for minor attacks during the period of study.

Case 6.—The patient, a 22-year-old man, developed asthma at the age of 16 months. His symptoms were perennial and fairly severe until the age of 14 years, when they eased considerably for a period of three years. Since then he has had considerable difficulty, although at the time of the study he was relatively free of symptoms. Physical examination showed a somewhat emphysematous appearance to the chest, which was also suggested by the x-ray picture.

There were occasional expiratory rhonchi to be heard over the lung fields. Skin tests were positive to several foods and inhalants, and symptoms were believed to be due to extrinsic factors.

In Fig. 8 are shown the pulmonary function tests in this case. It will be noted that during the eight-day control period symptoms were not particularly troublesome. The vital capacity was greater than predicted. The residual volume was slightly elevated and there was a considerable increase in the total capacity. The R.V./T.C. ratio was slightly greater than normal and the index of intrapulmonary mixing was within normal limits. There was a gradual increase in the maximum breathing capacity to nearly normal values.

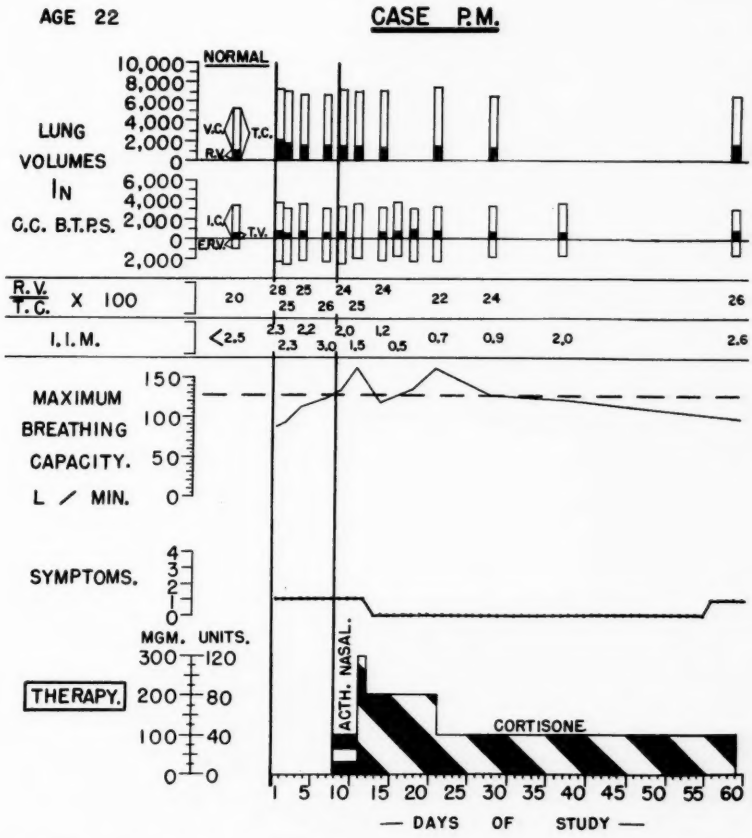


Fig. 8.—The effect of corticotropin and cortisone therapy on the pulmonary function of a 22-year-old man with extrinsic bronchial asthma. For explanation of symbols, see Fig. 1.

Despite the relatively normal findings in this case, an attempt was made to eliminate the remaining symptoms with corticotropin and cortisone. Forty units daily of corticotropin was administered for three days by intranasal insufflation and this was followed by oral cortisone. The intranasal corticotropin had no effect, but with the institution of cortisone the remaining symptoms disappeared.

There were no remarkable changes to be seen in the vital capacity, residual volume, or total capacity, although slight changes toward normal were noted. The R.V./T.C. ratio did not change appreciably, but the I.I.M. dropped to quite low values at times during the period of therapy. The maximum breathing capacity continued to rise and attained values above normal.

Case 7.—The patient was a 58-year-old woman with a history of chronic cough for many years and bronchial asthma for the past twenty years. Her symptoms were perennial and associated with considerable cough, but only

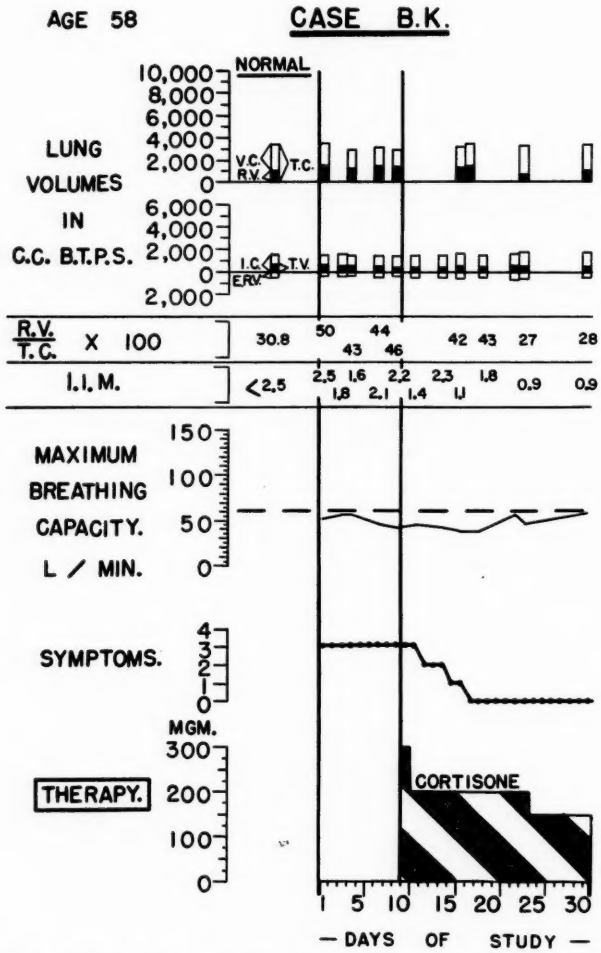


Fig. 9.—The effect of cortisone therapy on the pulmonary function of a 58-year-old woman with intrinsic bronchial asthma. For explanation of symbols, see Fig. 1.

rarely did she have any sputum. Physical examination revealed a somewhat barrel-shaped chest, with many expiratory rhonchi over the lung fields. The chest roentgenogram suggested emphysema, and skin tests were essentially negative. This patient was considered to have intrinsic asthma.

The results of the pulmonary function tests in this case are shown in Fig. 9. On admission to hospital there were moderately severe symptoms which continued unabated during the control period, despite frequent injections of adrenalin. The vital capacity was reduced and the residual volume was increased. The total capacity was essentially normal, but the R.V./T.C. ratio was considerably elevated (46; normal, 30.8). The index of intrapulmonary mixing was within normal limits and the maximum breathing capacity was not significantly reduced. These abnormal values persisted throughout the entire nine-day control period.

On the tenth hospital day the patient was started on oral cortisone. The symptoms began to decrease and by the eighth day of treatment she was symptom-free and remained so for the remainder of the period of study. The vital capacity did not show much change until the symptoms disappeared, but then it increased to predicted normal values. The residual volume showed a similar change and was well within normal limits at the end of the study. The total capacity did not change markedly. The R.V./T.C. ratio reflected the change seen in the residual volume, the value falling from 46 to 27. No particular changes were seen in the index of intrapulmonary mixing and maximum breathing capacity.

Case 8.—The patient, a 29-year-old man, had a history of frequent colds during childhood, followed by asthma at the age of 20 years. At first his symptoms occurred from spring to fall, but in recent years they became perennial and were associated with frequent respiratory infections. For the period immediately prior to this study, he was having only occasional attacks. Physical examination revealed a normal chest, and expiratory rhonchi could be heard only on exertion. The roentgenogram did not suggest the presence of any emphysematous changes. The skin tests were markedly positive to a wide variety of foods and inhalants and his symptoms were considered to be due to both extrinsic and intrinsic factors.

The pulmonary function tests obtained in this case are shown in Fig. 10. The patient had only slight respiratory difficulty on admission, which promptly cleared and did not return during the period of study. The vital capacity was normal in volume, but it was noted that the mid-capacity was higher than normal. The residual volume was only slightly increased, although the total capacity was much larger than normal. The R.V./T.C. ratio was not particularly abnormal. The index of intrapulmonary mixing was within normal limits and the maximum breathing capacity rose well above the predicted normal during the control period.

These results were not particularly abnormal and did not suggest the presence of much bronchospasm. However, it was decided to determine the effect of relatively large doses of corticotropin. A surprising degree of improvement was seen with this therapy. The vital capacity showed a rise to higher values. The residual volume decreased considerably and the total capacity was reduced by 1,500 c.c. The R.V./T.C. ratio reflected these changes and fell to 16, a value well below the predicted normal of 20. The index of intrapulmonary mixing

was reduced to very low values and the maximum breathing capacity showed a further considerable rise. It was evident that, despite the absence of symptoms and the relatively normal pulmonary function tests seen in the control period, there was present an appreciable degree of subclinical bronchospasm, as indicated by the improvement which followed the corticotropin therapy.

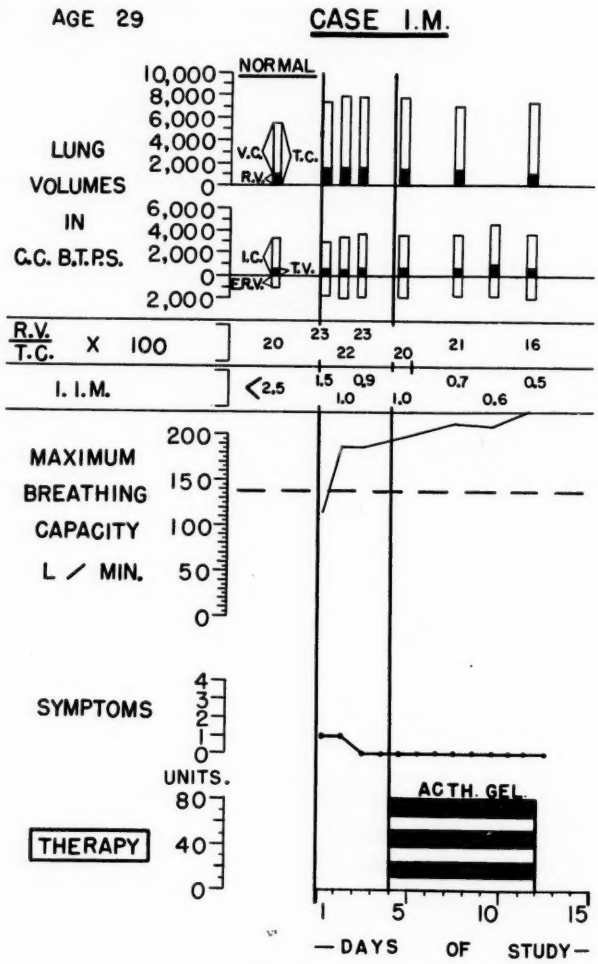


Fig. 10.—The effect of corticotropin therapy on the pulmonary function of a 29-year-old man with bronchial asthma. For explanation of symbols, see Fig. 1.

DISCUSSION

The degree of improvement seen in the pulmonary function of the eight patients studied, while variable, was striking. Patient 1, who was the most severely ill, showed the most remarkable changes. At the end of fourteen weeks' therapy, the pulmonary function had returned to normal. The residual volume, which initially was greater than 6 liters fell to 1,300 c.c. The x-ray changes

during the first five weeks of therapy, illustrated in Figs. 2 and 3, demonstrate that the emphysematous appearance of the chest regressed a considerable degree. An unusual finding in this case was the very large tidal volume associated with a slow respiratory rate. This occurred shortly after therapy was started and continued throughout the period of study. It is tempting to suggest that there existed an impairment of pulmonary elasticity which would require a longer period of time to return to normal. In the absence of measurements of the elasticity, however, no definite conclusions can be drawn. It will be noted also that when the dosage of hydrocortisone was reduced to such a level that severe symptoms recurred the pulmonary function tests returned, within eleven days, to values comparable with those found in the control period. Symptomatic improvement, however, was followed by a much more rapid return to normal values than had occurred in the previous remission.

There are two possible reasons for this. Asthma of long duration with an overdistended lung might be associated with a fatigue of the elastic components of lung parenchyma. Once these are allowed to regain their initial status by relieving bronchospasm and consequent air-trapping, their elastic properties are more readily regained following a second, but relatively short, stretching process. It is also probable that the edema and thickening of the bronchial mucosa associated with long-standing asthma are much less when an exacerbation of symptoms occurs as a result of lowering the effective dose of hormone therapy.

The second patient, despite freedom from asthma for a considerable period of time, did not show any change in pulmonary function. The explanation of this result is not readily apparent, but it may be due to the permanent structural changes of pulmonary emphysema or to subclinical bronchospasm relatively resistant to the usual hormonal dosage.

Patient 3 showed initial improvement in the pulmonary function during the control period associated with a spontaneous decrease in his symptoms. This improvement was continued during the treatment period and the vital capacity and index of intrapulmonary mixing returned to normal. The residual volume, total capacity, and maximum breathing capacity were still abnormal at the end of the study. However, during most of the period that the patient was receiving cortisone outside hospital there were minimal symptoms of asthma. It was noted that a large daily dose of cortisone was required to maintain him symptom-free. This dosage was associated with the development of side effects and it was not possible to maintain him at this level. In view of the persistence of low-grade symptoms, it is surprising that the pulmonary function improved to this degree.

Somewhat the same difficulty was encountered in Case 4. However, despite low-grade symptoms, the pulmonary function tests returned to normal values with the exception of the residual volume. While it did not fall to the predicted normal value, the R.V./T.C. ratio was well below values usually seen in chronic emphysema.[9]

Despite previous hormonal therapy, the pulmonary function tests in Case 5 were quite abnormal during the control period. With treatment there was a return to normal values, with the exception of the residual volume. The maxi-

mum breathing capacity was not considered to be a reliable test in this patient. The residual volume was still elevated at the end of the study, but was not at emphysematous levels. Again, periodic attacks of asthma were noted during treatment.

Patient 6 was relatively free of symptoms at the time of the study and did not show the gross abnormalities of pulmonary function seen in other cases. However, the slight changes that were found could be modified by therapy and returned to normal values.

Patient 7 showed a complete return to normal pulmonary function values within three weeks of beginning therapy. The eighth patient showed relatively normal pulmonary function during the control period, within the exception of the residual volume which was slightly elevated. With therapy, however, there was improvement in all the pulmonary function tests and the residual volume returned to normal values within ten days.

It is realized that the reliability of the results obtained from pulmonary function tests of the nature employed in this study is considerably dependent on the aptitude and cooperation of the patient. This is particularly true for the vital capacity and maximum breathing capacity and, to a lesser extent, for the index of intrapulmonary mixing. As will be seen from the relatively frequent determination, each of the eight patients in this study was thoroughly familiarized with the procedures in order to increase the reliability of the determinations.

In all of the cases studied a pattern of improvement could be followed. This consisted of an early return to normal of the vital capacity and maximum breathing capacity. The initially elevated mid-capacity, which is a reflection of "trapping" in asthmatic patients, soon fell, as did the index of intrapulmonary mixing. However, the index of intrapulmonary mixing fell to very low levels under the influence of hormone therapy and the reason for this is not apparent from our present data. It is possible that, under the influence of corticotropin, pulmonary function may increase beyond what is considered to be the normal predicted value. An example of this may be seen in Fig. 10, where the maximum breathing capacity rose well above the predicted value for that patient. In contrast to the rapidity with which these changes were reversed and symptoms suppressed, it required a somewhat longer period for residual volumes to revert toward normal and in some cases a considerable period of therapy was necessary before much change was evident. This was particularly true if there were symptoms during the treatment period.

In some of the cases difficulty was encountered in regulating the dosage of cortisone. It was found that complete removal of symptoms required large enough daily doses to produce undesirable side effects, such as excessive fluid retention and increase of acne. In these patient it was decided to decrease the cortisone, with a resultant improvement in the side effects and the development of occasional symptoms. In these cases the residual volume did not always return to normal and it is likely that better results would have been obtained if symptoms could have been completely relieved over a longer period of time.

Of the eight cases studied, four showed normal pulmonary function at the end of the study, three were normal with the exception of the residual volume,

and one showed no change. The three patients who demonstrated an incomplete return to normal continued to have symptoms during the treatment period which could not be removed without the development of cortisone side effects. However, there was a considerable degree of improvement in these cases and the values obtained at the end of the study were well below those normally seen in chronic emphysema. It is entirely possible that complete removal of symptoms with larger doses of corticotropin or cortisone would have resulted in a further fall in the residual volume. Case 2 showed no change in pulmonary function and this may have been due to the permanent parenchymal lung changes of chronic emphysema.

It would appear that in most cases of bronchial asthma the defect in pulmonary function is reversible. The bronchoconstriction may be removed more or less completely by the use of hormonal therapy with corticotropin, cortisone, or hydrocortisone. The more complete the removal of symptoms with such therapy, the more complete is the return to normal pulmonary function. Most of the patients studied had clinical symptoms when first seen, but two of them did not. In these two the improvement in pulmonary function seen with therapy was probably due to the removal of subclinical bronchospasm. The changes seen in this study suggest that in most instances long-standing bronchial asthma does not lead to the permanent changes of pulmonary function seen in chronic emphysema. The abnormalities of pulmonary function in bronchial asthma would seem to be due to chronic hyperinflation and are reversible with adequate therapy.

SUMMARY

The effect of long-term therapy with corticotropin and cortisone on the pulmonary function of eight patients with bronchial asthma has been studied. Four patients showed normal pulmonary function at the end of the study, three were normal with the exception of an elevated residual volume, and one showed no change. In three cases in which the residual volume remained elevated, symptoms of asthma were only incompletely relieved. It was found that there was a rapid improvement in the vital capacity, maximum breathing capacity, and index of intrapulmonary mixing with treatment but the residual volume required somewhat longer periods of therapy to return to normal. It is believed that the changes in pulmonary function found in bronchial asthma are due to hyperinflation of the lungs occasioned by the bronchospasm encountered in this disease and that, with treatment sufficient to completely remove the bronchospasm, these changes are reversible.

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STUDIES IN SENSITIZATION AS APPLIED TO SKIN TEST REACTIONS

III. Influence of Age Upon Skin Reactivity

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IN A previous communication,[1] we discussed the various factors which might influence skin test reactions. It was pointed out that positive reactions may increase, decrease, or remain stationary, depending upon such influences as avoidance, specific treatment, or various physiologic reasons. The latter included change in the reactivity of the skin with advancing age. Thus, as indicated by our questionnaire survey,[1] some allergists believe that older people have a lessened skin reactivity which accounts, perhaps, for the change in skin reactions upon retest. Clinical experience also has suggested that there is a lower incidence of positive skin test reactions in the aged, even to potent allergens, but these are opinions based solely upon clinical impressions rather than upon the results of investigative studies. This is supported by a review of the literature which did not reveal any confirmatory evidence to support these contentions. In an attempt to obtain more definite evidence, investigations were carried out, the results of which form the basis of the present report.

METHOD OF STUDY

Two types of studies were undertaken. The first consisted of the determination of the incidence of positive skin test reactions at various decades of life; the second involved the determination of skin reactivity to varying concentrations of histamine at varying age periods.

To obtain the information comprising the first part of this study, we reviewed the skin test records of our private patients to determine the incidence of positive reactions to food allergens at various ages. Starting with the letter "A," we went through our files alphabetically, recording the age of the patient and the skin reaction, until we had obtained a total of 100 for each food and for each decade. The foods selected were those listed as "commonly eaten" and "uncommonly eaten" and utilized in a previously reported study[2] concerning the contact factor in skin sensitization. Listed as commonly eaten foods were banana, chicken, chocolate, corn meal, egg, flounder, lobster, milk, orange, oyster, green pea, peanut, strawberry, tomato, wheat; the uncommonly eaten ones were blackberry, chestnut, goose, green pepper, sweet potato, pumpkin, and rhubarb.

From the Allergy Clinic, Temple University Hospital and School of Medicine.
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All tests were done by the intracutaneous method, 0.02 ml. of the routine dilution of the food extract being injected. Only those positives were selected which had been retested at least once to be certain of their specificity. Reactions were classified either as negative or as slight, moderate, or marked positive; doubtful reactions were considered negative. The totals for each decade and for each grade of reaction are listed in percentages in the tables.

For the second part of this study, routine intracutaneous skin tests (0.02 ml.) were performed with four dilutions of histamine (1-1000, 1-10,000, 1-100,000, and 1-1,000,000) upon nonallergic persons at various age periods, selecting 100 persons for each decade of life. The results were recorded as negative, slight, moderate, or marked. All doubtful reactions were considered negative. An attempt was made to carry out similar tests upon allergic patients, both private and clinic, currently being studied. We were unable to obtain a number in the various decades comparable to that of the nonallergic group, so the evidence furnished by this portion of the study has been omitted from the present study.

RESULTS

A summary of the data comprising the results of the first part of the investigation is tabulated in Tables I and II and charted in graphic form in Figs. 1 and 2. It will be noted in Table I that we were able to obtain 100 records

TABLE I. COMMON FOODS. INCIDENCE OF POSITIVE REACTIONS ACCORDING TO AGE GROUP

AGE GROUP	TOTAL NUMBER TESTS	PER CENT NEGATIVE	PER CENT SLIGHT	PER CENT MODERATE AND MARKED	PER CENT TOTAL POSITIVE
0 to 10	1,500	75.3	22.1	2.6	24.7
11 to 20	1,500	78.5	19.9	1.6	21.5
21 to 30	1,500	81.0	17.3	1.7	19.0
31 to 40	1,500	79.3	19.3	1.4	20.7
41 to 50	1,500	80.9	18.2	0.9	19.1
51 to 60	1,500	86.2	13.1	0.7	13.8
61 to 70	817	86.8	12.7	0.5	13.2
71 and over	172	87.2	12.2	0.6	12.8

TABLE II. UNCOMMON FOODS. INCIDENCE OF POSITIVE REACTIONS ACCORDING TO AGE GROUP

AGE GROUP	TOTAL NUMBER TESTS	PER CENT NEGATIVE	PER CENT SLIGHT	PER CENT MODERATE AND MARKED	PER CENT TOTAL POSITIVE
0 to 10	700	91.0	8.9	0.1	9.0
11 to 20	700	93.2	6.6	0.2	6.8
21 to 30	700	92.9	7.1	0	7.1
31 to 40	700	93.8	6.1	0.1	6.2
41 to 50	700	95.0	5.0	0	5.0
51 to 60	700	95.6	4.4	0	4.4
61 to 70	277	97.8	2.2	0	2.2
71 and over	63	96.8	3.2	0	3.2

for each of the fifteen common foods, giving a total of 1,500 tests for each decade up to the age of 60 years. After age 60, however, the total for each food was less because of the lack of patient material in that age period. This also was true for each of the seven uncommon foods; 100 records for each one were

obtained up to the age of 60, giving a total of 700 for each decade, but a smaller number was obtained in those after 60 (Table II). Because of the smaller number present in the groups aged 61 to 70 years and 71 years and over, the totals in these two age periods were combined when the graphs were made. These slight deviations, however, did not interfere with either the final results or their interpretation.

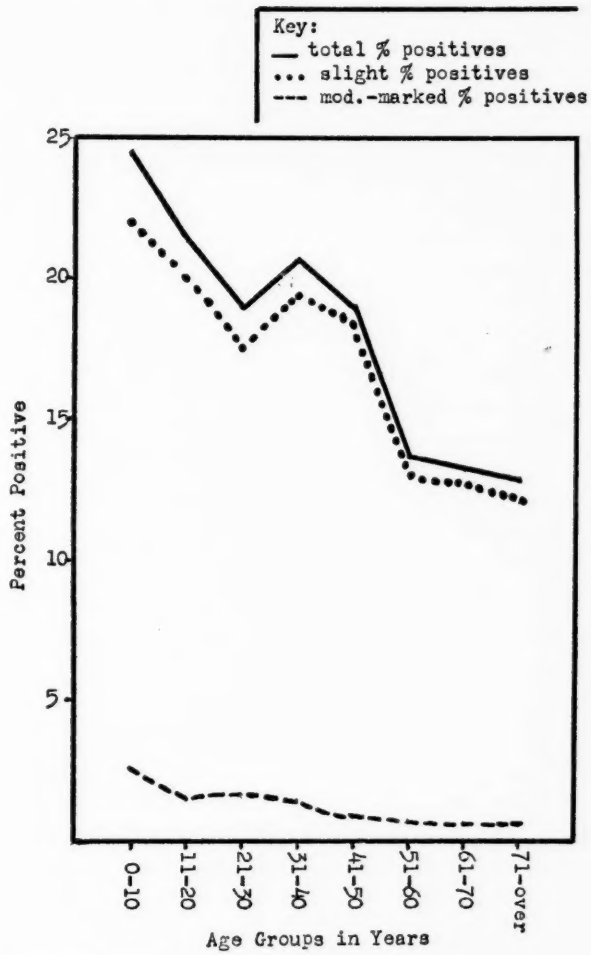

Fig. 1.—Incidence of positive reactions to common foods in allergic patients according to age group.

As seen from the tables, and more graphically in Figs. 1 and 2, the incidence of positive reactions is greatest in the first decade, with only slight reduction in the second decade. Little change then occurs between the ages of 21 and 50 years, but from 51 to 60, and especially after 60, there is a sharp drop in incidence, particularly when one compares it with the first decade. This is true not only of the composite curves (Fig. 1, solid line) but also of

the individual curves for slight positive reactions (dotted line). The percentage for the moderate-marked positive reactions (combined together, as there were so few marked positives) was too low for accurate interpretation, but even these curves show a diminishing tendency with advancing age (broken line). The diminishing incidence of positive reactions with advancing age is seen more strikingly in the curves of the commonly eaten foods

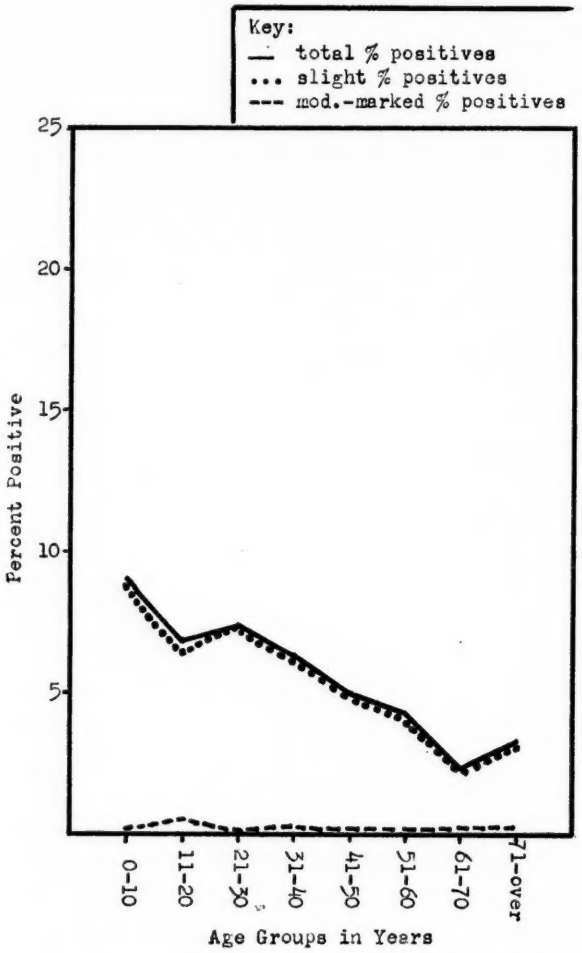

Fig. 2.—Incidence of positive reactions to uncommon foods in allergic patients according to age group.

(Fig. 1) than in those of the uncommonly eaten ones (Fig. 2), although the general contour of the curve is similar in both. However, as indicated in our previously reported study,[1] this is because the incidence of positives in the uncommonly eaten group ordinarily is low so that little change is to be expected. This is verified also by the extremely low and almost negligible number of moderate to marked positives in the uncommon group as compared

with the commonly eaten foods. On the whole, the results of this portion of the investigation serve to verify the clinical impression that skin reactivity is highest in the first two decades, and that a slight decrease then occurs in the third, fourth, and fifth decades, with definite diminution after the age of 50 years.

The results of the tests with histamine in nonallergic persons at various decades are shown in Table III and Fig. 3. Inspection of these shows at once that nearly all persons, regardless of age, reacted to histamine in the 1-1,000 concentration, in either moderate or marked degree. On the other hand, few reacted to the 1-1,000,000 concentration, even in slight degree. This renders the latter concentration unsuitable for use in interpretation for comparative purpose, as contrasted particularly with the results of the tests with the other three strengths. Likewise, since in the 100 tests with 1-100,000 concentrations there were only eight moderately positive and no marked positive reactions, these results are included for purposes of comparison with the slight reactions as "positive" without regard to degree. Similarly the four marked positive reactions to the 1-10,000 strength are included with the moderates, whereas the eight negatives are grouped with the slights and the slights with the moderates in the 1-1,000 strength, both for purposes of comparison and for charting the curves.

TABLE III. INCIDENCE OF SKIN TEST REACTIONS TO HISTAMINE IN NONALLERGIC PERSONS*

CONCENTRATION OF HISTAMINE	REACTION	AGE GROUPS							
		0 TO 10	11 TO 20	21 TO 30	31 TO 40	41 TO 50	51 TO 60	61 TO 70	71 AND OVER
1-1,000,000	Negative	88	98	100	99	97	100	100	100
	Slight	12	2	0	1	3	0	0	0
	Moderate	0	0	0	0	0	0	0	0
	Marked	0	0	0	0	0	0	0	0
1-100,000	Negative	6	22	11	16	16	10	38	39
	Slight	87	78	88	84	84	90	62	61
	Moderate	7	0	1	0	0	0	0	0
	Marked	0	0	0	0	0	0	0	0
1-10,000	Negative	0	0	1	2	0	0	0	5
	Slight	15	42	25	28	40	34	61	61
	Moderate	85	58	74	70	59	63	39	34
	Marked	0	0	0	0	1	3	0	0
1-1,000	Negative	0	0	0	0	0	0	0	0
	Slight	0	2	2	6	3	6	10	15
	Moderate	25	37	52	36	64	64	69	73
	Marked	75	61	46	58	33	30	21	12

*One hundred tests for each decade with each concentration.

In making an interpretation of the results, it will be seen from inspection of Table III that in the 1-100,000 concentration, there is a progressive increase in negative reactions from the first decade on, compared with a progressive decrease in slight positive reactions. Thus, the reactivity is highest in the first decade (94.0 per cent positive) and lowest both in the sixth decade (62 per cent) and after age 71 (61 per cent). Incidentally, it is to be noted as verification of the high reactivity of the first decade that practically all the moderate reactions (seven) to the 1-100,000 strength occurred in this age

period. This progressive reduction of reactivity, as indicated by the slight positives, is illustrated in the broken line shown in Fig. 3. Similar findings were obtained with the results of the tests with the 1-10,000 concentration. Thus, there is a progressive increase in the slight positive reactions from the first decade (15 per cent) to those after age 60 (61 per cent) and, conversely, a progressive decrease for the moderate positive reactions from 85 per cent

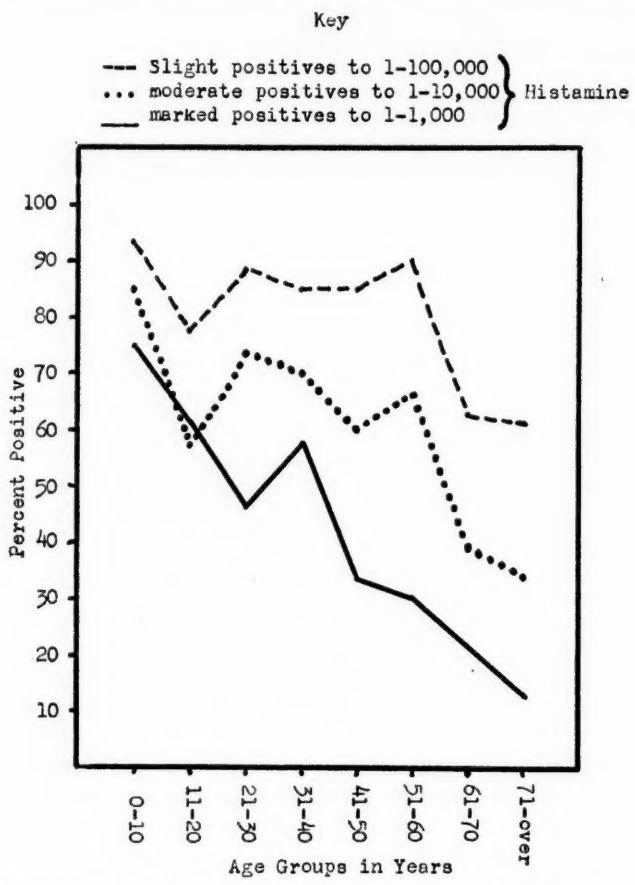

Fig. 3.—Incidence of skin test reactions to histamine in nonallergic patients.

in the first decade to 34 per cent in the group past 70 years of age, which also showed the largest number of negative reactions (5 per cent). This decrease in reactivity is shown in Fig. 3 by the dotted line representing the moderately positive reactors. Finally, these findings also are verified by the results with the tests to the 1-1,000 concentration which show an increase in moderate reactions and a corresponding decrease in marked positive reactions from the first decade to the period after 70 years of age. The latter is represented by the solid line in Fig. 3.

Regardless, therefore, of which concentration is utilized, the results as shown graphically in the curves in the chart indicate that, at least in non-

allergic persons, the reactivity of the skin in relation to histamine is highest in the first decade. It diminishes thereafter, slightly and not altogether uniformly, from the second to the fifth decades, inclusive, with little tendency to any consistent or striking diminution. But after age 60, there is a definite and marked reduction in reactivity, the curve falling rather precipitously from ages 61 to 70, with little reduction after age 71. The results of these histamine tests roughly parallel those obtained from the analysis of the incidence of positive allergic skin test reactions according to age as shown in Fig. 1. The only point of difference is that in the latter a sharp drop in incidence as seen in the curve occurs after age 50, whereas in the histamine group it does not appear until after age 60. Whether this is the result of statistical variation, perhaps ascribable to the inclusion in the older age group of patients likely to have few or no positive reactions (as, for example, those with the nonallergic or "intrinsic" forms of asthma) or whether it represents an actual difference in the reactivity of the skin of the allergic patient, as contrasted with the nonallergic person, awaits further elucidation.

COMMENT

The demonstration of a reduction in skin reactivity with advancing age provides an additional reason, as indicated in our first report,[1] for changes in skin test reactions, especially when patients are retested at long intervals. The actual reason for this decrease is not known, but for the present it must be regarded as physiologic and normal.

Realization of this loss of reactivity in the skin of older people may be important clinically, because until now we have assumed that failure to obtain positive reactions with routine tests, especially in persons past 50 years of age, indicated an absence of sensitization to specific allergens. As a result, such persons often are classified as being nonsensitive or nonallergic. The results of the present study suggest that failure to react may be due to a low threshold of skin reactivity rather than to an absence of specific sensitivity. Since patients usually are tested with that concentration of the allergenic extract which has been considered from experience to be the proper testing strength, older persons with lessened reactivity might show a negative reaction with that concentration but a positive one when tested with the next higher strength. We see this not infrequently in hay fever patients, some of whom may react only to higher concentrations (0.01 or 0.1 mg. of N per milliliter) and others to lower ones (0.0001 or 0.001 N). Unless this variation in skin reactivity is realized, a positive test may be missed. Since most people react to histamine in a 1-10,000 or 1-100,000 concentration, routine skin tests with these concentrations might provide a clue as to the patient's skin reactivity. Thus, if patients have a slight reaction to the 1-100,000 and a slight to moderate reaction to the 1-10,000 strength, their reactivity can be considered average or normal. If, however, they react negatively to the former and doubtfully or only slightly to the latter, their reactivity should be considered relatively low. This should be taken into account in the interpretation

of their skin tests. We are now utilizing these tests in our allergy patients for the estimation of their skin reactivity in the hope that this experience might determine its clinical usefulness.

SUMMARY AND CONCLUSIONS

1. The incidence of positive skin reactions to food allergens is highest in the first decade of life and decreases slightly up to age 50, but after 50 (and especially after 60) there is a sharp decrease, especially when compared with the first decade.

2. Skin tests with histamine in varying concentrations done on nonallergic persons at different decades of life show that skin reactivity is highest in the first decade and diminishes slightly from the second to the fifth decades, with definite and marked reduction after the age of 60 years.

3. Failure to demonstrate positive reactions in older persons may be due to loss in skin reactivity rather than to a lack of specific sensitivity.

4. The possibility of utilizing histamine skin tests (1-10,000 and 1-100,000) for determining skin reactivity is discussed.

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FUNGUS COLONY COUNTS FROM AIR-BORNE SPORES IN BIRMINGHAM, ALABAMA

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THE air-borne spores of Alternaria and Cladosporium have received considerable attention as inhalant allergens. The allergist should be informed as to the variety of spores in a local area, since many fungus spores have been incriminated as allergy incitants. The purpose of this investigation was to determine the kinds and prevalence of air-borne fungus spores in Birmingham, Alabama, by the culture plate method. A yearly count was undertaken in an effort to obtain information on the seasonal fluctuation of the fungi encountered and to attempt an interpretation of the data obtained in relation to precipitation, relative humidity, and wind direction.

The advantages of plate cultures over direct mold spore slide counts are many. Colony counts and concurrent slide spore counts are seldom in agreement.[1] Only those molds possessing relatively large spores or spores with marked characteristics can be recognized by the slide method. Alternaria spores, which are large and typical, and Cladosporium spores, which usually occur in clumps, can be identified readily. Aspergillus, Penicillium, and others, the spores of which are relatively small and indeterminant, are more difficult, if not impossible, to identify. By the use of an artificial medium supporting growth of the majority of air-borne fungi, positive identification following incubation of the exposed plates is usually possible. Although culturing with consequent identification of genera is more time-consuming, it is generally recognized as a more accurate method than that of slide spore counting.

The survey covered the year from June 1, 1953, to May 31, 1954. Three exposures a week of three plates each for a five-minute period were made. Following development of colonies, usually in five to seven days, results were tabulated, unidentifiable genera were isolated for further study, and the monthly total of each recognizable genus was compiled. Species identification was not undertaken.

Birmingham, situated at the foothills of the Great Smoky Mountains, has an elevation of between 500 and 1,000 feet. The average monthly rainfall during the survey was 3.3 inches. The average relative humidity was 66.6 per cent, and the average temperature was 63.2 degrees. Since the rainfall and humidity were not excessive, abundance of the Phycomycetes was not anticipated.

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PROCEDURE

Exposure of Sabouraud's plates to the air was made after trial exposures of malt agar, potato-dextrose agar, and Sabouraud's dextrose agar. Sabouraud's agar was found to satisfy the criterion of a favorable medium, since it was easy to prepare, supported the growth of the common air-borne fungi, limited bacterial growth, and was a medium on which the common mold genera were easy to recognize. Pigmentation of certain colonies and reverse coloration were readily apparent.

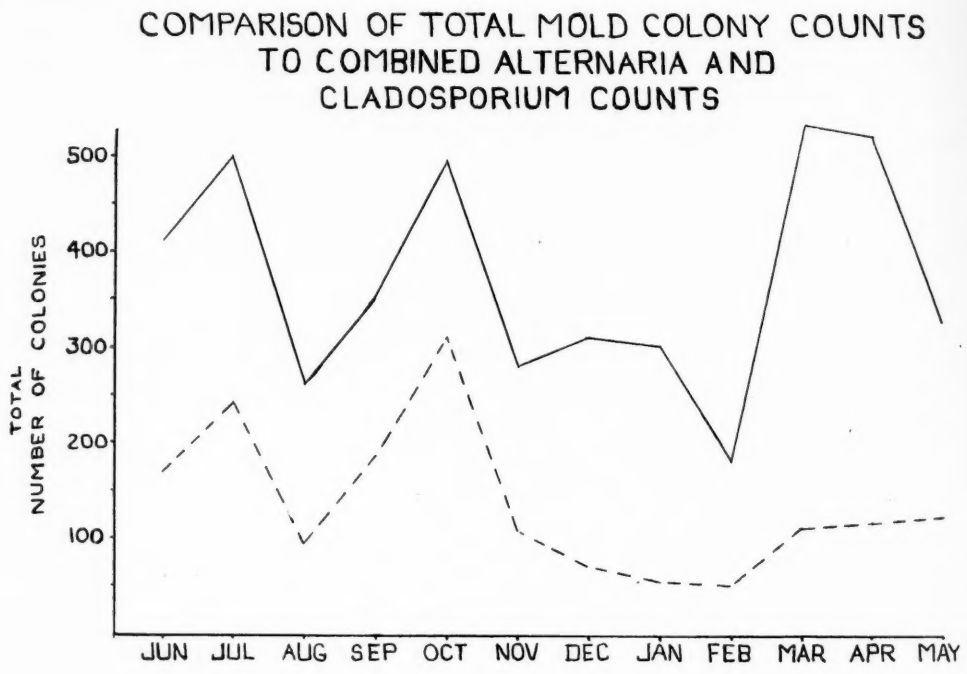

Fig. 1.

Numerous types of media have been employed by other investigators.[2-4] Since there seemed little likelihood, from a cursory observation of trial exposures and general climatic conditions, that many spreading Oospora, Aspergillus, and Rhizopus or other Phycomycete colonies would be found which might overgrow on the plates, the choice of Sabouraud's medium was made.

Each Monday, Wednesday, and Friday midmornings three plates were exposed for five minutes on a brick ledge on the roof of the Medical Center Building. Previous test exposures indicated that five minutes was the maximum exposure time for eventual maturation of discrete colonies. There were 156 exposures of three plates each, or the equivalent of 468 separate exposures.

Plates were incubated at room temperature, undisturbed, for five to seven days, after which time colonies were identified macroscopically, and microscopically by use of cotton blue moist preparations. Unrecognizable colonies were isolated and further identification attempted.

DISCUSSION

Fig. 1 shows the total mold colony counts by months, compared to the combined total Alternaria and Cladosporium spore counts. Those two mold colonies were found throughout the entire year, comprising, roughly, one-third of all colonies counted. The summer season and on through October showed the heaviest fall of both of these spores. The greatest number of these colonies was recorded in October. From November through March, the Alternaria and Cladosporium counts remained consistently lower, and a slow rise occurred in April and May approaching the summer peak season.

The total number of colonies counted during August was low, as were the counts of both Alternaria and Cladosporium. The prevailing wind that month was from the southwest, a dry arid region where normal counts of these two molds are low.[5] Eleven days of rain may have been instrumental in washing spores from the air.

Table I shows the distribution of the nine most frequently encountered molds, the miscellaneous mold distribution, the total mold colony count, the unidentified molds, and the meteorologic data for the year. The genera, Alternaria, Cladosporium, Stemphylium, and Acrothecium, belong to the Dematiaceae and Botrytis, Penicillium, and Aspergillus are Moniliaceae, whereas Phoma is classed in the family Sphaeriodaceae, and Epicoccum in the family Tuberculariaceae. Of the total of 4,488 colonies counted, 174 were not identified. No marked seasonal variation was observed for Penicillium, Aspergillus, Acrothecium, or Epicoccum. During June a high Stemphylium count was recorded, and a shower of Phoma with a record of 195 colonies on April 30 may have occurred as a result of a strong wind from the south on that day. Heavy falls of Botrytis during December, January, and March occurred on rainy days when the wind was from the south.

Combinations of climatic factors undoubtedly influenced daily spore dissipation. In general, Alternaria and Cladosporium were more prevalent when the prevailing wind was from the west. Rain temporarily washed spores from the higher atmosphere, and counts were lower following precipitation. Plates exposed during the rain often developed increasing numbers of Pullularia and Cryptococcus colonies.

Alternaria and Cladosporium spores, although more prevalent in the summer, were found during the entire year. Winds from the north and midwest may well have brought some of these spores from the wheat-growing areas during the height of the wheat season. Since these two molds were found consistently during the year, they must have a natural habitat and maturation in this area.

The remaining seven mold genera, while demonstrating no seasonal fluctuation, were recovered during each month and may be considered as potential allergens. However, the quantity of air-borne spores of any genus does not in itself incriminate that fungus as an allergy incitant.

TABLE I. MONTHLY MOLD COLONY COUNTS AND LOCAL CLIMATOLOGICAL DATA, JUNE 1953 TO MAY 1954

GENUS	JUNE	JULY	AUG.	SEPT.	OCT.	NOV.	DEC.	JAN.	FEB.	MARCH	APRIL	MAY	TOTAL	PER CENT OF TOTAL	YEARLY AVERAGE
Cladosporium	96	120	40	94	209	57	48	32	31	81	89	67	964	21.5	
Alternaria	65	117	51	91	101	50	25	22	20	32	30	57	661	14.7	
Botrytis	8	95	17	24	11	19	101	106	21	281	55	25	763	17.0	
Penicillium	25	25	9	36	25	18	33	60	30	17	18	13	309	6.9	
Phoma	5	8	4	5	2	2	24	10	9	6	204	14	293	6.5	
Stemphylium	125	22	23	19	18	10	8	10	4	9	19	12	199	6.2	
Acrothecium	9	17	23	23	24	13	4	5	2	6	9	15	150	3.3	
Aspergillus	4	2	8	6	8	24	30	8	5	2	5	20	122	2.7	
Epicoccum	6	7	6	4	9	7	4	14	7	20	17	24	125	2.8	
Total miscellaneous colonies	65	76	72	52	75	65	31	36	43	68	70	75	728	16.3	
Unidentified	5	11	6	9	11	15	6	3	6	11	6	5	174	2.1	
Total count	413	500	259	363	493	280	314	306	178	533	522	327	4488	100	
Average temperature (degrees)	81.4	79.7	79.5	74.0	65.2	51.6	43.4	46.2	51.2	53.4	67.2	65.1			63.2
Total precipitation	1.22	4.71	2.80	2.98	0.21	1.62	9.04	6.35	3.37	3.51	3.20	3.23			3.33
Number of rainy days	7	11	11	4	2	5	17	10	6	9	11	8	101		
Average relative humidity	65.8	73.0	66.3	61.1	61.0	59.5	71.9	72.1	60.1	72.7	68.9	66.7			66.6
Wind direction	NE	N	SW	NE	W	SE	NW	SW	SW	SW	NW	NW			

TABLE II. INCIDENCE OF AIR-BORNE FUNGI

	(PER CENT)		(PER CENT)
I. FUNGI IMPERFECTI			
A. *Dematiaceae (Dark to black hyphae and/or spores)*		B. *Moniliaceae (Conidia hyalin or bright-colored, usually on conidiophores)*	
Cladosporium	21.5	Botrytis	17.0
Alternaria	14.7	Penicillium	6.9
Stemphylium	6.2	Aspergillus	2.7
Acrothecium	3.3	Monilia	1.5
Pullularia	1.3	Trichoderma	1.4
Helminthosporium	0.5	Oospora	1.2
		Paecilomyces	0.6
Miscellaneous—less than 0.5 per cent each		Miscellaneous—less than 0.5 per cent each	
Curvularia		Cephalosporium	
Montospora		Scopulariopsis	
Nigrospora		Fusidium	
Mycogone	0.8	Trichothecium	
Synsporium		Verticillium	1.2
Stachybotrys		Geotrichum	
Mesobotrys		Gliocladium	
Spondylium		Botrytrichum	
		Cylindrocarpon	
Per cent of total	48.3	Per cent of total	32.5
C. *Stilbaceae (Conidiophores bound into coremia)*		D. *Tuberculariaceae (Fruiting layer of hyphae and conidiophores colored, intercalary chlamydospores)*	
Stysanus	0.05	Fusarium	1.7
		Epicoccum	2.8
Per cent of total	0.05	Per cent of total	4.5
E. *Sphaerioidaceae (Pycnidia dark, membranous)*		F. *Melanconiaceae (Conidiophores in superficial or erumpent stratum, conidia may have appendages)*	
Phoma	6.5		
Chaetomella	0.4	Pestalozzia	0.1
Per cent of total	6.9	Per cent of total	0.1
II. PHYCOMYCETES			
A. *Mucoraceae (Columellate sporangia, nonseptate hyphae, zygospores)*			
Rhizopus	0.8		
Per cent of total	0.8		
III. MISCELLANEOUS			
A. *Streptomycetaceae (Hyphae usually not more than 1.5 μ in diameter, white or colored, conidia in chains)*		B. *Saccharomycetaceae (No mycelium, asci, loosely attached spores)*	
Streptomyces	1.3	Sacchromyces	0.5
		C. *Cryptococcaceae (No mycelium, after budding spores)*	
		Cryptococcus (red-orange)	2.3
Per cent of total	1.3	Per cent of total	2.8
D. *Mycelia-Sterilia (indefinite or globose sclerotium)*	0.6	E. *Unidentified*	2.1
Per cent of total	0.6	Per cent of total	2.1

Table II is a composite tabulation of the total percentage of each mold genus found during the year. The Phycomycetes account for only 0.8 per cent of all the colonies counted. The Fungi Imperfecti comprised 92.4 per cent of the total; 80.8 per cent of these belonged to the families Dematiaceae (48.3 per cent) and Moniliaceae (32.5 per cent). The remaining 6.8 per cent

were miscellaneous or nonsporulating, unrecognizable genera. Cryptococci, Streptomyces, and Saccharomyces comprised only a small percentage of the recorded colonies.

SUMMARY

A total of 4,488 colonies of air-borne fungi was recorded in a one-year period from 468 plate exposures.

The Phycomycetes (0.8 per cent) and the Fungi Imperfecti (92.4 per cent) composed 93.2 per cent of all the identified colonies counted.

Cryptococci, Saccharomyces, Myceelia-Sterilia, and unidentifiable fungi made up the remaining 7.6 per cent of the colonies counted.

Alternaria and Cladosporium accounted for 36.2 per cent of the total colonies, showing the heaviest production during June, July, September, and October.

No marked seasonal variations were found among the remaining genera.

Showers of Botrytis and Phoma occurred during a number of months of the year.

At least nine mold genera occurred in quantities sufficient to be incriminated as possible allergens.

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CLINICAL EVALUATION OF A NEW ANTIHISTAMINE, BUCLIZINE HYDROCHLORIDE (VIBAZINE*)

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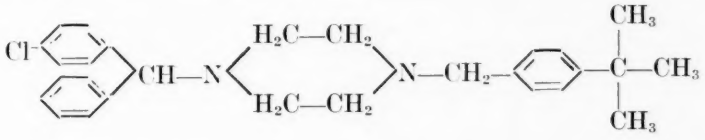
DURING the decade or so since the innovation of the first histamine-antagonizing agents, numerous compounds representing structural modifications of the original substances have been devised. The aim has been to produce compounds that (1) exhibit a greater degree of effectiveness in a higher percentage of the various allergic disorders, (2) are attended with a lower incidence of untoward reactions, and (3) have a longer duration of action and thus obviate the inconvenience of frequent administration.

Many of the effective compounds have an ethylenediamine group in their structure,[1] and fairly recently one with a diethylenediamine (piperazine) group was introduced. This latter agent is chlorcyclizine[2] and it is characterized by a relatively prolonged duration of action (eight to twenty-four hours).

A more recent and particularly promising piperazine derivative has become available for clinical trial and differs from chlorcyclizine by having three, rather than two, benzylic rings in its structure.[3, 4] The purpose of this article is to report a clinical trial of this new compound, buclizine hydrochloride, which has been named "Vibazine" by the manufacturer and is designated chemically as 1-*p*-chlorobenzhydryl-4-*p*-(*tert.*-butyl)-benzylpiperazine. It is represented by the following structural formula:



Pharmacologic studies demonstrated that orally administered doses of Vibazine protected guinea pigs from nebulized or intravenously injected histamine for at least four days in all cases, a period of protection that was considerably longer than was provided by even larger doses of either tripelennamine or chlorcyclizine. The oral $L.D._{50}$ of Vibazine in mice was found to be 2,100 mg. per kilogram of body weight, while that of tripelennamine was 235 mg. per kilogram and that of chlorcyclizine was 500 mg. per kilogram. Chronic toxicity studies in rats and dogs receiving the drug over periods of six months revealed no morbid changes. These data indicate high effectiveness, low toxicity, and a prolonged duration of action for Vibazine.

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*Vibazine hydrochloride (brand of buclizine hydrochloride) was kindly supplied for this study by Dr. M. Carlozzi of Chas. Pfizer & Company, Inc.

MATERIALS AND METHODS

In this study, Vibazine was used in the adjunctive treatment of fifty-nine patients with various allergic disorders, principally seasonal hay fever. The patients included twenty-three males and thirty-six females, ranging in age from 4 to 61 years; ten of them were children 12 years of age or younger. The individual dose found effective in most cases was 25 mg., but an individual dose of 50 mg. was found to give better results in six (10 per cent) of the fifty-nine patients. The effective dose was given one to six times daily, as determined by the response in the individual case (only once or twice daily in 50 per cent of the patients). The duration of therapy ranged from one day (five patients) to five weeks of continuous use (eight patients). The average duration was two weeks.

RESULTS

The therapeutic results achieved with Vibazine are tabulated in Table I. "Excellent" results represent 75 to 100 per cent relief; "fair" results represent partial, but less than 75 per cent, relief; and "poor" results represent little or no relief.

THERAPEUTIC RESULTS WITH VIBAZINE IN FIFTY-NINE PATIENTS*

CONDITION	NUMBER OF CASES	RESULTS		
		EXCELLENT	FAIR	POOR
Hay fever	53	29	13	11
Vasomotor rhinitis or non-seasonal coryza	10	6	1	3
Bronchial asthma	6	3	2	1
Urticaria	2	2	—	—
Vernal conjunctivitis	1	1	—	—
Totals	72	41	16	15

*Some patients had more than one allergic condition.

It will be seen from these figures that 80 per cent of the patients were benefited, 60 per cent obtaining excellent relief. Only 20 per cent were not relieved. It is noteworthy that three of the fifteen patients not relieved had previously used other antihistamines without benefit.

One of the patients obtaining an excellent result stated that Vibazine was the most effective antihistamine of any that he had used, another stated that it was the only one that he had found effective at all, and nine patients (seven with excellent results and two with fair results) found it superior because it was the only one without side effects.

Onset of Action.—Relief appeared in one-quarter to one-half hour after administration in all fifteen patients in whom this factor was determined.

Duration of Action.—In the forty-six patients benefited by Vibazine, the duration of relief after a single dose was at least twelve hours in twenty-two (50 per cent) and as long as twenty-four hours in twelve. The duration of relief was no longer than eight hours in ten patients, no longer than six hours in

eight, and three or four hours in six. Thus, in the majority (70 per cent) the duration of action was sufficiently long to last through the night after a bedtime dose.

Side Effects.—Adverse reactions occurred in six (10 per cent) of the patients. The most frequent was drowsiness (four patients); headache was reported by one, and jitteriness by one. It was necessary to discontinue use of Vibazine in three cases (5 per cent of patients treated), because of drowsiness in one, jitteriness in one, and headache in one. The patient who discontinued Vibazine because of drowsiness had obtained an excellent therapeutic effect, but was unable to tolerate any antihistamine. The patient experiencing jitteriness also had excellent relief, but had a similar reaction to all antihistamines she had tried. One patient with drowsiness not severe enough to require discontinuance of Vibazine had excellent relief and had previously found other antihistamines valueless. Another with drowsiness not severe enough to require discontinuance of Vibazine had fair relief and reported having experience the same reaction to other antihistamines. The third with drowsiness not severe enough to require discontinuance of the drug had fair relief but required individual doses of 50 mg.

Ten patients who previously had been unable to tolerate other antihistamines because of the drowsiness they produced were able to use Vibazine without side effects. Of these, seven had excellent results (and in one Vibazine was the only antihistamine found effective), two had fair results, and one had a poor result (all other antihistamines had also proved ineffective).

SUMMARY AND CONCLUSIONS

A new antihistaminic drug, buclizine hydrochloride (Vibazine hydrochloride) was submitted to clinical trial in fifty-nine patients with various allergies, principally hay fever. A significant degree of relief was provided in 80 per cent of the cases treated, the results being excellent in 60 per cent. The following observations drawn from this study indicate that Vibazine represents a valuable addition to the list of histamine-antagonizing agents.

1. Vibazine compares favorably in effectiveness with the most potent of such agents in current use. In fact, several patients found it to be the only one that provided relief.

2. As regards side effects, Vibazine compares favorably with the best tolerated antihistamines. Untoward reactions severe enough to require discontinuance of its use occurred in only 5 per cent of the series reported, and nine patients unable to tolerate any other antihistamines were relieved by Vibazine without side effects.

3. Vibazine provides the advantages of prolonged action. In 70 per cent of the patients, relief was maintained for eight to twenty-four hours after a single dose. This means that the action of a bedtime dose will last through the night in most cases.

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American Academy of Allergy

REPORT OF THE COMMITTEE ON DRUGS OF THE RESEARCH COUNCIL OF THE AMERICAN ACADEMY OF ALLERGY, 1954-55

DIPHENYLPYRALINE

AN ANTIHISTAMINIC drug, Diphenylpyraline, supplied and manufactured by Schenley Laboratories, Inc., was studied by the following members of the Committee on New Drugs: Drs. Murray Albert, Theodore Bernstein, Sidney Friedlander, Harold Friedman, Edward Keeney, Dean Moffat, Irving Schiller, Aaron Spielman, Walter Winkenwerder, and Carl Arbesman.

The results (with the exception of two reports) were tabulated on special IBM cards, and various cross relationships were determined. This drug was given to 316 patients and the data obtained are listed in Table I. Of the 273 patients with seasonal allergic rhinitis, 38 per cent of them had very adequate relief of symptoms. However, only 25 per cent of the patients with perennial allergic rhinitis were benefited. The symptoms of rhinorrhea, sneezing, and itching were alleviated more readily than was nasal occlusion. Diphenylpyraline was ineffective in bronchial asthma, and only relatively beneficial in urticaria.

TABLE I. SCHENLEY'S DIPHENYLPYRALINE (316 PATIENTS)

CONDITION	NUMBER OF PATIENTS	PERCENTAGE OF PATIENTS IMPROVED*
Seasonal allergic rhinitis	273	38
Perennial allergic rhinitis	24	25
Bronchial asthma	6	0
Urticaria		
Acute	4	75
Chronic	6	33
Atopic eczema	2	50
Contact dermatitis	1	100

*Only those patients having 50 per cent or more relief of symptoms were classified as improved.

This drug, when effective, gave relief within thirty minutes in 88 per cent of the patients (Table II). However, the action of the drug lasted only one to four hours in 75 per cent of the patients who responded well. Beneficial results for seven or more hours were recorded in 7.8 per cent (Table III).

The incidence and severity of the side effects from this drug are illustrated in Table IV. As with most other antihistaminics sedation was the side effect most frequently encountered. Moderate to severe ill effects were reported in 7.5 per cent of the patients.

TABLE II. SCHENLEY'S DIPHENYLPYRALINE TIME OF ACTION (163 CASES)

LENGTH OF TIME TO BECOME EFFECTIVE (MINUTES)	NUMBER OF CASES
15 to 30	143 (88%)
45 to 60	20 (12%)

TABLE III. SCHENLEY'S DIPHENYLPYRALINE. DURATION OF ACTION (156 CASES)

LENGTH OF TIME EFFECTIVE (HOURS)	NUMBER OF CASES
1 to 2	43 (27.6%)
3 to 4	75 (48%)
5 to 6	26 (16.6%)
7 or more	12 (7.8%)

TABLE IV. SCHENLEY'S DIPHENYLPYRALINE. SUMMARY OF INCIDENCE OF SIDE EFFECTS*

Total number of patients	238	
Total number of side effects	70	29.4%†
Total number of patients with side effects	54	22.6%‡
Total number of moderate to severe side effects	22	9.2%
Number of patients with moderate to severe side effects	18	7.5%

*From IBM cards. One investigator reported without cards (fifty-one patients).
†Eight (16 per cent) patients with all degrees of side effects.
‡Ten (20 per cent) total side effects.

TABLE V. SCHENLEY'S DIPHENYLPYRALINE. TYPES AND INCIDENCE OF SIDE EFFECTS

TYPES	SEVERITY				
	MINIMAL	SLIGHT	MODERATE	SEVERE	TOTAL
Lethargy, sleepiness	21	11	7	6	45
Dizziness	5	4			9
Nausea	1				1
Dryness of mouth	2		3	2	7
Headache		1	1		2
Insomnia	1			1	2
Nervousness				1	1
Eye Swelling			1		1
Palpitation	2				2
Total	32	16	12	10	70

TABLE VI. RESULTS OF DIPHENYLPYRALINE IN SEASONAL ALLEGRIC RHINITIS (38 PER CENT IMPROVED)*

INVESTIGATOR	PERCENTAGE OF PATIENTS IMPROVED*
Albert	23
Arbesman	40
T. Bernstein	49†
Friedlander	51
Friedman	25
Moffat	50†
Schiller	48
Spielman	42
Winkenwerder	8

*More than 50 per cent improvement of symptoms.
†Not from IBM cards.

Conclusions.—Diphenylpyraline (Schenley) is a fairly active antihistaminic drug. It compares quite favorably with many of the other antihistaminics already available, but has no particular advantage over them. The percentage of side effects was quite low, however, and this drug should have merit in patients who cannot tolerate other antihistaminics.

PLIMASIN

An antihistaminic combined with an analeptic was supplied to this committee through the courtesy of Ciba Pharmaceutical Products, Inc. This product, called Plimasin, contains 25 mg. of Pyribenzamine and 5 mg. of Ritalin in each tablet. Ritalin's chemical formula is phenyl-piperidyl-(2)-acetic acid methyl-ester. This synthetic compound has a marked psychomotor-stimulating effect, and its action would be classified as somewhere between caffeine and amphetamine.

The purpose of this survey was threefold: (1) to note whether or not the 25 mg. dose of Pyribenzamine was adequate, (2) to observe the effect of the addition of Ritalin in the Elimination of the side effect of sleepiness, fatigue, and lethargy, and (3) to note any synergistic effect of Ritalin and Pyribenzamine on the allergic symptoms.

Method.—Supplies of three different tablets, all of which looked alike, were sent to the various members of the Committee on New Drugs. One tablet was Plimasin; another, 25 mg. of plain Pyribenzamine; and the third, 5 mg. of Ritalin alone.

The following members of the committee have already sent in their findings: Friedlaender, Friedman, Joyce, Moffat, Schiller, Spielman, Albert, Keeney, and Arbesman.

This present report is essentially a preliminary survey, as most of the committee members did not have these drugs long enough or during a pollen season. The committee hopes to continue this study and report in more detail at a later date.

The results presented here include the reports of the committee members just mentioned, plus those of Dr. Samuel Feinberg who has had an opportunity to evaluate this compound for a longer period of time.

Results.—A total of 192 patients who were given Plimasin were finally available for study. This group plus Dr. Feinberg's 89 patients made a grand total of 281 patients. The dosage varied from one pill twice daily to as many as two pills four times daily.

The results of clinical effectiveness of Plimasin, Pyribenzamine, and/or other antihistaminics and Ritalin are listed in Table VII. As can be seen, Plimasin is an effective antihistaminic.

TABLE VII. CLINICAL EFFECTIVENESS

CONDITION*	DRUG USED	NUMBER OF PATIENTS	PER CENT IMPROVED
Allergic rhinitis	Plimasin	250	68
	Pyribenzamine‡	113	74
	Ritalin	44	16
Urticaria	Plimasin	17	53
	Pyribenzamine	11	37
	Ritalin	3	0
Dermatitides§	Plimasin	12	50
	Pyribenzamine	4	50

*One case of bronchial asthma and one of migraine were not helped by Plimasin.
†Fifty per cent or more improvement of symptoms.
‡Pyribenzamine or other antihistaminics.
§Including contact dermatitis, atopic dermatitis, and pruritus of unknown etiology.

The relative effectiveness of Plimasin and Pyribenzamine or other antihistaminics used is illustrated in Table VIII. Of the 199 patients who received both of these drugs at different times, 124 felt that they were equally beneficial, 47 thought that Plimasin was better, and 28 claimed that Plimasin was not as effective as the other antihistaminics.

TABLE VIII. COMPARATIVE EFFECTIVENESS OF PLIMASIN WITH OTHER ANTIHISTAMINICS IN SAME PATIENTS

1. Better than others	47
2. Worse than others	28
3. Same as others	124
	Total 199

The side effects of the available antihistaminics are well known. Drowsiness or sedation is the most common reaction encountered. Of the 128 patients who received Pyribenzamine or other antihistaminics, 63 patients complained of sedation. However, only 11 of 192 patients treated with Plimasin were sleepy (Table IX). Stimulation of the central nervous system, as evidenced by jitteriness, nervousness, insomnia, palpitation, dizziness, depression, and headache, was more prevalent with Plimasin than with Pyribenzamine. In most instances, however, this was due to an increased dosage of the Plimasin (two tablets per dose) or to ingestion of the drug in the evening. The side effects of sleepiness, lethargy, fatigue, weakness, and depression are all grouped under sedation.

TABLE IX. SUMMARY OF SIDE EFFECTS

TYPE	DRUG USED			
	PLIMASIN	PYRIBENZAMINE*	RITALIN 5 MG.	RITALIN 10 MG.
	(Total Number of Patients)			
	192	128	35	23
Sedation	11	62	--	--
Central stimulation	39	13	11	11
Gastrointestinal	19	10	--	--
Number of patients with side effects	58	80	12	11
Number of patients without side effects	134	48	23	12
Total number of moderate to severe side effects	47	70	12	6

*Pyribenzamine or other antihistaminics.

The nausea, emesis, dry mouth, diarrhea, and bad taste are classified in the gastrointestinal side effect (Table IX).

The comparison of Plimasin's side effects with other antihistaminics, particularly Pyribenzamine, in the same patient was made in 163 instances (66 from Dr. Feinberg). The data are listed in Table X. As can be seen from this information, Plimasin greatly reduces the side effect of drowsiness.

TABLE X. COMPARISON OF PLIMASIN'S SIDE EFFECTS WITH OTHER ANTIHISTAMINICS

Better than others in (less drowsiness)	103 patients
Same as others	36 patients
Worse than others (central nervous system effect)	17 patients

Summary and Conclusion.—Plimasin is an effective antihistaminic and produces a minimum of side effects. The usual sedation seen with other antihistaminics is almost absent. This makes this compound a useful drug in the treatment of allergic rhinitis and urticaria. The central stimulating effect of this drug was considerably more frequent than with Pyribenzamine or other antihistaminics. This fact, although not alarming, would indicate that the drug should not be prescribed in the evening or upon retiring or in larger dosages (more than two tablets per dose), even in the daytime.

Carl E. Arbesman,
Secretary, Committee on Drugs
Research Council
American Academy of Allergy

Announcement

New York Medical College Offers Postgraduate Course in Pediatric Allergy

A postgraduate course in pediatric allergy will be held at the New York Medical College, Flower and Fifth Avenue Hospitals, under the direction of Bret Ratner, M.D., Professor of Clinical Pediatrics and Associate Professor of Immunology, from Nov. 2, 1955 to May 31, 1956, (thirty sessions) on Wednesdays, 9 A.M. to 4 P.M. The fee is $300.00; enrollment is limited.

The course consists of lecture-seminars, laboratory and clinical procedures, clinic work, ward rounds and animal experimentation covering basic principles of diagnosis and treatment of allergy in children, and applied immunology.

Applicants must be certified in pediatrics or have the requirements for certification. Apply to: Office of the Dean, New York Medical College, Fifth Avenue at 106th St., New York 29, New York.

A research fellowship in pediatric allergy is available. Application should be made now.

Book Reviews

Reactions With Drug Therapy. By Harry L. Alexander, M.D., Philadelphia and London, W. B. Saunders Company, 1955, 301 pages.

This book summarizes, in a form convenient for reference, a large volume of literature dealing with drug reactions. The basis for the often difficult distinction between hypersensitivity and hypersusceptibility to toxic effects is discussed, and it is with idiosyncracies of the former type that the book is concerned. The first portion of the book describes the more common types of reactions and lists the drugs known to produce each. The second portion deals with the various drugs and the reactions that each may cause. Tests and precautions that help to foresee or prevent reactions in some cases are described, as is the treatment of the more serious type of reactions. It should be of considerable value in helping the physician to recognize promptly the complications of drug therapy and, in the case of patients receiving a multitude of drugs, to guess the probable cause of a specific manifestation of idiosyncracy.

Since the handling of the subject is primarily practical, the distinction between hypersensitivity and toxicity is perhaps too sharply drawn. Thus, the discussion of reactions to quinine seems incomplete with no mention of cinchonism and that of reactions to streptomycin seems incomplete with no reference to eighth nerve disturbances. Likewise carbon tetrachloride and tetrachloroethylene are omitted from the list of drugs causing hepatitis. Obviously, these may all be properly classed as manifestations of toxicity, which occur regularly with excessive doses, but the practitioner is more directly concerned with the possibility that they occasionally may be produced by relatively small doses than with their theoretical classification.

On the other hand, in a field where books are so few, many readers would welcome a more complete discussion of the mechanisms of the phenomena described, more extensive use of structural formulas of the drugs, description of their breakdown in the body, and their reaction with protein to form the complex antigens which are assumed to be the basis of drug allergy.

Regional Allergy of The United States, Canada, Mexico and Cuba. A Symposium of Thirty-nine Contributors, edited by Max Samter, M.D., and Oren C. Durham, Springfield, Ill., Charles C Thomas, 1955, 395 pages. Price, $8.50.

While the practicing allergist is presumably familiar with the pollens, molds, and other local allergens peculiar to his own area, he is sure to have occasional need of similar information concerning other parts of the continent in order to advise patients who may be contemplating a change of residence either for their health or for other reasons. The present book provides this material for convenient reference. All the contributors are recognized authorities on the practice of allergy in their areas, but differ considerably in their enthusiam for botany. The latter factor no doubt influences the number of different pollens considered important in various areas.

The editors have allowed the authors a free hand in presenting the subject, and the results have, in general, justified their confidence. However, the book would be improved if uniform seasonal charts of pollination were included in all chapters. The discussions of regional allergens are by no means limited to pollens and molds. Local industrial allergens,

regional foods, and insects receive consideration. Surprisingly, the plant causing the highest incidence of sensitization in most areas, *Rhus toxicodendron,* is mentioned by few of the contributors.

The geography, climate, industry, and social and economic characteristics of each area are also described, giving added information of interest to a patient considering a change of residence. While most of the authors seem pleased with their locations, the accounts of climate are restrained and factual.

Formulario di Aerosolterapia (Formulary of Aerosol Therapy). By Sergio Rocchietta, Edizioni Minerva Medica, p. 444.

The third edition of a most interesting manual, *Formulary of Aerosol Therapy (Formulario di Aerosolterapia),* by S. Rocchietta, has recently been released by the Italian press.

The present manual, following the first and second editions in short order, is in itself a tribute to its popularity and usefulness, and, by the same token, need for revision and augmentation attests to the increasing interest in the expanding field of aerosol therapy.

This volume, which is twice the size of the previous edition, is divided into four parts:

Part one, on aerosol therapy, includes a general discussion, classification of drugs, preparation of solutions, solvents, vehicles, etc.

Part two is in the form of a dictionary of the drugs used to date in aerosol therapy, alphabetically listed and individually described as to preparation, action, etc.

Part three discusses those drugs which may be used without harmful effect on people, objects of value, etc., in disinfecting the atmosphere.

Part four covers research in aerosol therapy and discusses the action, absorption, and elimination of aerosols, experimental tuberculosis in rabbits produced for study purposes by inhalation of bacilli of human and bovine types, radioactive aerosols, radiopaque substances, allergenic extracts, etc.

Because of the variety of subjects covered, this manual has appeal to many members of the profession.

FEDELE L. BONITO, M.D.